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EXPERIMENTAL MEDICINE

FIBRINOUS EXUDATES AND FIBRINOID DEGENERATION.

BY H. R. GAYLORD, M. D.

(*From the Pathological Institute of Professor Orth, University of Gottingen.*)

PLATES I AND II.

No subject in pathology has been the cause of more discussion or of more radically divergent interpretation of its phenomena by scientific investigators than that of fibrinous exudates on serous surfaces.

The literature of the subject previous to 1880 shows a fairly even division of opinion. The investigators of this period form two groups, one of which believed that the fibrin was the result of exudation and coagulation, while the other attributed its presence wholly or in part to degeneration of the connective tissue forming the groundwork of the serous membrane. To mention briefly and in their chronological order the authorities who have adhered to the theory of exudation, we must begin with Cohnheim (4), who describes the classical exudation of fibrin on serous surfaces and its organization by connective tissue. Of the epithelium of the serous membrane he speaks clearly, stating that it either becomes swollen and undergoes fatty degeneration, or exceptionally through proliferation of connective tissue is dislodged, and thus robbed of nutrition, perishes.

After Cohnheim, Wagner (6) studied the question and described clearly the characters of the fibrin, which, he says, consisted of a network of fibres in which large, glistening, homogeneous clumps were nearly always present. The pleural epithelium he found in fresh, as well as older, inflammations intact below the fibrinous deposit. In closing

he declares for the older view that the fibrin originates in exudation from the circulation.

Weigert (7) supports the theory of exudation, and speaks of the clumps and masses of fibrin found in the fibrinous network. Later he says (8) that the endothelium of the pleura must be destroyed to produce such an exudate, and that such a destruction of endothelium marks the boundary between tissue and fibrin.

Orth (14) accepts the theory of exudation and coagulation. The cases coming under his observation showed the usual network and the homogeneous masses. The epithelium, according to his observations, disappears early in the process, first showing proliferation of the nuclei and swelling and granulation of the protoplasm. After the disappearance of the epithelium the fibrin lies directly upon the connective tissue of the pleura.

The first to oppose the theory of exudation was Rokitansky (1). He believed that the fibrin covering serous membranes was the result of tissue "vegetation." The connective tissue first proliferated and then became a hyaline mass without structure. The new tissue obtained its nutriment partly from the vessels to be found in it, and partly from the fluid contents of the serous cavity.

Virchow (2), in his Gesammelte Abhandlungen, says: "The raw, dry, uneven fibrinous membrane is in such intimate connection with the underlying tissue that it is at times as difficult as in the case of diphtheritic membranes to determine a sharp boundary between tissue and fibrin. One merges so gradually into the other that the whole gives at once the impression that the fibrin is nothing more than degenerated tissue." Two years later he again (3) expressed himself as opposed to the theory of exudation and said that the fibrinous mass is in part nothing more than degenerated and swollen tissue.

Buhl (5), although not excluding the possibility of the fibrin having its origin in exudation, considered that the solid portions of the mass were produced by the pleural connective tissue through a process of proliferation and inflammation, and he called the product "desmoider Faserstoff." He differentiated two layers of the same, a deep and a superficial layer. The former contained blood-vessels and cells in large number, was a direct continuation of the pleural connective tissue, and found its limit always at the outermost elastic lamella. The superficial layer was free from vessels and amorphous in character, and he could occasionally demonstrate endothelial cells covering its free surface. The cases examined by Buhl were clearly, from the above description, in an advanced stage of organization.

Neumann (9), in an article on the use of picrocarmine staining in the study of inflammatory conditions, came to the conclusion that in tubercular inflammation the fibrinous masses were the result of degeneration of connective tissue. He agreed with the view of Buhl in so far as the origin of the fibrin was concerned, but considered the process a passive one and named it "fibrinoid degeneration" of connective tissue. In tuberculosis he could demonstrate the change from connective tissue to fibrin in all its stages. He found that the connective tissue first became swollen and lost its fibrillar appearance. From the red-staining connective tissue there developed shining masses which the picric acid stained yellow. The masses were often isolated in the connective tissue, but on the surface became fused together, giving the appearance of an exudation. He found epithelium on the surface but rarely, and then only in small, isolated patches. In acute pleuritis associated with croupous pneumonia and in acute purulent puerperal peritonitis he was of opinion that the deeper layers of the fibrinous mass were the result of fibrinoid degeneration. He did not, however, apply the idea of degeneration to acute cases free from connective tissue proliferation, but considered them more probably the result of exudation.

The authors quoted above, who made special investigations of the subject from 1855 to 1880, left the question but slightly changed in its general aspects. As technique improved, much of the work of older investigators who had opposed the theory of exudation was disproved, and the last quoted investigation of Neumann, which closed this first period, left the scientific world either adhering to the old theory of exudation or taking the middle stand advanced in his article.

From 1880 till 1892 the question preserved much the same status, although during this time two writers on the subject, Langhans (13), in an article on periorchitis sero-fibrinosa and pleurisy with fibrinous deposit, and Schuchardt (21), in an investigation of subacute hygroma, came to the conclusion that the fibrin was produced by a degenerative process in the connective tissue.

In 1892, through an article published by Schleiffarth (19), a pupil of Grawitz, and one year later by statements made by Grawitz himself (17), the question received a new impetus. Grawitz and Schleiffarth claim that the fibrin is the result of degeneration of connective tissue. Schleiffarth states that the tissue degenerates in one layer after another, and lays no stress on the position of the elastica. Neither of them agrees with Buhl and the last named investigators that the fibrin is produced directly from the connective tissue. Grawitz (18) advances the theory that there is an intermediate stage in which he can see cells and nuclei

develop from the connective tissue and from the lymph spaces of the same, and these he calls "slumbering" cells. This idea of the development of cells from the tissue constitutes the so-called "Schlummerzellen-Theorie" of Grawitz. He believes that the cells which other observers call leucocytes are not always to be seen as such, and that the protoplasm from which they are produced can become fibrin, or that their nuclei, when formed, can remain enclosed in the fibrinous mass and form the cell elements found in fibrinous exudates. As proof of this theory, so far as its application to fibrinous exudates is concerned, Grawitz and Schleiffarth cite the regular arrangement of the cells and nuclei in the fibrinous mass, which lie parallel to the direction of the connective tissue of the serosa, and the possibility of demonstrating the epithelium or the outermost fibrous constituents of the serosa to be intact, while beneath them bands of fibrin can be found. At other times the fibrin present on the surface can be demonstrated as the result of a direct degeneration of the connective tissue.

Following the article of Schleiffarth, Apel (29), a student of Orth, published a dissertation embodying the study of twenty-eight cases of simple acute and specific inflammations of serous membranes. His conclusion was that the middle position taken by Neumann was correct. For specific cases he accepted a degeneration of connective tissue, but for acute cases he considered the fibrin the result of exudation alone. In all his acute cases (15) he found the epithelium unbroken beneath the fibrin. In specific cases he found degeneration and exudation combined. He cited a case of cancerous peritonitis on which a laparotomy had been performed during life and from which the operator had removed a large quantity of fluid which coagulated. Upon microscopic examination of specimens from this case, Apel found that beneath the fibrin covering the surface the connective tissue in many places showed the degeneration described by Neumann, and he concluded that both processes, exudation and degeneration, can occur together. As to the work of Schleiffarth and Grawitz, Apel says that in his specimens he was unable to find any appearances which could possibly suggest the development of cells and nuclei from the intercellular substance of connective tissue.

In a review of Grawitz's atlas Marchand (22) says: "One of the most remarkable acquisitions to modern science, which makes its appearance in this atlas, is that fibrin found on the surface of serous membranes is not an exudate, but degenerated intercellular substance. The fibrinoid pseudo-membrane does not rest upon the pleura, but is the result of degeneration of the superficial layers of the same, in fact (in the intestine) this degeneration can extend through the entire thickness of the

muscularis. In short, the process is one of direct degeneration of connective tissue; otherwise how were it possible to account for the Schlummerzellen (alias leucocytes) found in the fibrin if they were not produced where they are found?" In support of his statement regarding fibrin, Grawitz gives photographs (Plate XXIX of his Atlas) in which Marchand says he can see the partly preserved epithelium underlying the mass. In closing Marchand says: "It is well known that in certain cases fibrin can be produced by exudation in the tissue itself: why not in the serosa?" "No one who had not a theory to support would confuse this condition with a pseudo-membrane, or on careful examination (without special staining methods) would fail to find the connective tissue fibres in the fibrin."

In answer to this criticism of Marchand, Neumann again entered the discussion in support of the theory of fibrinoid degeneration as applied by Grawitz and Schleiffarth, but he attempted to separate that phase of the question from the theory of slumbering cells, stating that the discussion involved "two questions, where the fibrin had its origin and whence came the cells found in it." Neumann now extends his theory, applied originally to chronic processes, to acute as well. This new position is based largely upon a case of acute pleurisy with fibrinous deposit, covering which he found a continuous layer of epithelium which he concludes is the pleural epithelium in its original anatomical position, and therefore that all fibrin below it is derived from degenerated connective tissue. He considers as characteristic of this degeneration the hyaline masses and bands found in the deeper layers of pseudo-membranes. These bands are, according to his investigations, nothing more than connective tissue fibres in partial degeneration, and represent a transitional state between the connective tissue below and the completely degenerated mass (fibrin) above.

In answer to this article of Neumann there appeared an exhaustive argument against it by Marchand (23), and an address on the subject before the pathological section of the Naturforscherversammlung in Frankfurt by Professor Orth, based upon the specimens of Vassmer and myself.

In reply, Neumann (12) published a short restatement of his position, laying special stress upon the points which he did not consider to have been satisfactorily met in Marchand's article. It is especially with these last two publications of Neumann (11 and 12) that the present article proposes to deal.

Neumann (12) says: "As fibrinoid degeneration of connective tissue, I have described a condition, in my experience widely distributed, in which the connective tissue degenerates, forming a mass which resembles

macroscopically and microscopically, and gives the same chemical reactions as, fibrin the result of coagulation of blood. This change is accomplished by the bundle of connective tissue swelling, and melting together in such a manner that fibres can no longer be recognized as such, the whole forming a highly refractive mass, in which can be seen at first the nuclei of connective tissue cells." Morphologically the change can be compared to amyloid degeneration in which the tissue undergoes a change forming amyloid substance, except that in the fibrinoid metamorphosis the connective tissue undergoes a degeneration the result of which is a mass identical with blood fibrin. The best examples of this degeneration, he says, are to be found in the study of inflamed serous membranes. In particular he cites that form of inflammation found in the pleura overlying portions of hepatized lung, and states that the mass of fibrin found in such cases is the result of such degeneration for the following reasons: first, he finds that the pleural epithelium covers the mass; second, a sharp boundary between the fibrin and the underlying connective tissue at the beginning of the process does not exist. The epithelium which may be found under the fibrin he agrees with Schleiffarth in explaining as endothelium of a lymph channel, which appears only after the process is well advanced and marks the boundary between active process and degenerated mass.

To this can be added a case of chronic fibrous periorchitis reported by Sultan (27), in which the mass of fibrin covering the tunica vaginalis lay between two continuous layers of epithelium, one covering the mass and the other at the point of demarcation between fibrin and tunica vaginalis. This case he explains by assuming that the epithelium covering the mass is the original epithelium of the tunica vaginalis which with the mass has been thrown off, while the layer of epithelium underlying the mass he believes to be the result of regeneration.

The point made by Neumann that at the beginning of exudative processes in serous membranes no cell demarcation exists, does not agree with my experience. A careful examination of all cases coming to hand in the Pathological Institute during the last ten months shows overwhelming evidence to the contrary. The fresher the condition the more perfectly preserved has been the epithelium, a statement which applies to simple and septic cases of pleurisy and pericarditis. Figs. 1, 2 and 3 (Plate I) are photo-micrographs of such cases and can leave no doubt as to the character of the cells. That such a cell demarcation is not referable to a collection of endo-

thelial cells in a lymph space seems to me to be conclusively proven by the following facts: The mass of fibrin lies directly upon the cells; there is no connective tissue between the row of cells and the fibrin, and in Fig. 1 (Plate I) the fibrin is separated from the cells which are clearly attached at their base. Moreover the cells appear continuous in all sections cut from the same block as well as from blocks taken from different parts of the pleura or pericardium, which could not happen if we were dealing with cells arranged in lymph spaces, in which case we would find intervening tissue. That these cells, as Neumann says, do not make their appearance until after the process is well advanced, and that they are necessarily associated with a cellular infiltration in the connective tissue of the serosa, is opposed by the appearances in Fig. 3 (Plate I) in which the exudate is very fresh and the connective tissue free from cellular infiltration. Vasmer (16), who made an exhaustive study, by means of orcein staining, of the relation of the elastica of the normal pleura and pericardium to the epithelial covering, found that the epithelium when not arranged directly over the elastica is separated from it by only a small layer of connective tissue, which in the pericardium is less than in the pleura.

From these facts it is safe to take the stand that where we find no change under the elastica it is very improbable that the small amount of connective tissue lying over it can have furnished the entirely disproportionate amount of fibrin so commonly observed. If, moreover, we find connective tissue between the elastica and the fibrinous mass, it seems evident that we require no cell demarcation to show that the fibrin has not been produced at the expense of the underlying tissue.

Neumann recognizes the relation of the fibrin to the outermost elastic lamella of the pleura and pericardium, and states that the process, *i. e.* fibrinoid degeneration, finds its limit at the elastica. Buhl also accepted the elastica as the boundary of the process. Schleiffarth, on the contrary, pays no attention to this well-known anatomical landmark, and simply says that the fibrin is the result of the degeneration of the connective tissue which extends inwards, one layer after another becoming degenerated.

In all cases where I was not able to demonstrate epithelium this relation of the elastica to the fibrinous mass has invariably shown the fibrin lying over the elastica, and in the majority of cases a distinct amount of connective tissue between the elastica and fibrin. In Figs. 1, 2, 3 and 4 (Plate I) the elastica is stained with orcein and shows clearly this relation. I cannot speak too strongly of the value of this method, as in many cases at first doubtful the staining of the elastica proved the only means of clearly determining the relation of the exudate to the underlying structures.

Neumann describes as characteristic of simple non-septic cases a fine fibrinous membrane, which is found at the very beginning of the process. Such membranes, he says, cannot be removed with the forceps without tearing away portions of the underlying tissue. These membranes he considers to be the very beginning of the process, before the fibrin has been thrown off and while it is still connected to the tissue, from the degeneration of which he supposes it to originate. It is in these cases that he finds no epithelium under the fibrin and states that a sharp line of demarcation between fibrin and connective tissue does not exist. Marchand (22), speaking on this point, says: "Every beginner knows that it is exactly the freshest fibrinous membranes which are the easiest to remove." My experience agrees with this statement of Marchand, except that the very fresh exudates, owing to their extreme thinness, could not be removed in large pieces. It was always in such cases that I found continuous epithelium under the mass. The case described by Neumann, in which the epithelium covered the fibrin, certainly did not present the condition usually found. The only other observer who distinctly describes the condition noted by Neumann is Buhl. He observed, in cases which we now know to have shown an advanced state of organization, small patches of epithelium covering the granulations.

Neumann says he has in a number of fresh cases found epithelium covering the exudate. His method for observing the same is as follows: The fibrin carefully removed from the fresh preparation is spread with its outer side upward on a glass slide, and then covered with salt solution. The specimen can be examined at once, or he recommends

staining it on the slide with acetic acid vesuvin, which gives the nuclei a deep brown, the fibrin a yellow color. All specimens coming to the Institute have been examined by this method, and, while I have here and there found cells such as Neumann describes, I have never failed to find on the under surface large patches of undoubted epithelium, and in many cases have been able to demonstrate in the hardened section continuous epithelium under the fibrin. I should consider the evidence obtained by this method exactly the opposite to that which Neumann claims for it.

In November, 1895, seven months before the appearance of Neumann's first article, I undertook, at the suggestion of Professor Orth, a series of experiments on animals, to ascertain if fibrin obtained from blood, when introduced into the serous cavities, would organize as such, and what microscopical appearances such specimens would present as compared with exudates in the human body. These experiments showed that fibrin, introduced into serous cavities in animals under aseptic conditions, will organize in the usual way in which fibrinous exudates organize, and presents at every stage of the organization the same processes which we find in the human body.

The scope of the experiments covered, first, the introduction of fibrin as such; and, second, the injection of coagulable fluid which produced the fibrin in the serous cavity. As fibrin, I introduced that obtained from whipping blood (slightly hardened in formalin) and coagulated blood plasma. As coagulable fluid, I injected plasma obtained from horses' blood, first with the addition of a fibrin ferment, and then alone.

Of the experiments, eighteen were successful and gave identical results. The results for the pleural and peritoneal cavities were the same, but the difficulty of finding the fibrin after introduction into the peritoneal cavity led me in my later experiments to use the pleural cavity alone. The first five experiments were made with fibrin obtained by whipping, and were so planned as to show the organization at different stages.

Experiment I.—Grey rabbit. Introduced into right pleural cavity two pieces of fibrin, size of small pea.

Autopsy seven days after operation. Incision shows no signs of infection. Pleura shiny. No fluid in pleural cavity. Fibrinous mass unchanged in appearance is found adherent to lower lobe of right lung at its outer inferior border. It appears to lie in an impression in the surface of the lung, and its base is surrounded by a secondary fibrinous exudate. Surrounding pleura shows no change.

Lower lobe of lung and fibrin hardened in Müller-formalin solution. Embedded in paraffine. Microscopic appearances. Section shows a large mass of fibrin adherent at its base to the parietal pleura. Pleural epithelium intact and shows evidences of proliferation at several places underlying the fibrin. Original mass of fibrin is surrounded by a secondary fibrinous exudate containing leucocytes, which also penetrate into the fibrinous mass proper. Lung tissue directly underlying the fibrin shows atelectasis. Fibrin, both that introduced and that secondarily formed, give the usual color stain with Weigert's method, and the yellow reaction with Neumann's modification of the picrocarmine stain.

Experiment II.—White rabbit. Introduction into right pleural cavity of two pieces of fibrin stained with aniline-water gentian-violet. (This experiment was made to ascertain earliest appearance of secondary exudate, hence the staining of introduced fibrin before introduction.)

Autopsy forty-eight hours after introduction. Both pieces of fibrin found adherent to costal pleura covering cartilages. Pleura shows a red zone 1½ mm. wide, surrounding base of fibrinous masses.

Costal cartilages, to which fibrin is adherent, dissected out and with adherent mass hardened in Müller-formalin. Embedded in paraffine. Microscopic appearances (picrocarmine). Fibrinous mass stained blue surrounded by secondary fibrinous exudate stained yellow. A few leucocytes can be seen in the exudate. Pleural epithelium beneath fibrin intact. At the sides of the preparation we have the pleural epithelium covered by the secondary exudate alone, giving the picture of a fresh exudate. Blood-vessels of the pleura distended with blood.

Experiment III.—Grey rabbit. Introduced into right pleural cavity two pieces of fibrin size of small peas.

Autopsy eight days after operation. Both pieces of fibrin found organized, being attached to costal pleura. Pleura at base of masses appears normal. Both pieces removed with intercostal tissue and hardened in Müller-formalin solution.

Microscopic examination. Entire fibrinous mass infiltrated with leucocytes. At base the mass is the seat of proliferation of connective tissue, which has organized the lower third of same. Fibrin of homogeneous character, except at base where we find glistening masses, many of which

are isolated in the newly proliferated connective tissue. Fine blood-vessels can be traced running from the superficial vessels of the pleura into the newly proliferated tissue. Pleural epithelium intact in patches underlying the mass, but cannot be demonstrated where we find the proliferation of connective tissue. Examination of the free surface of the mass shows it to be covered with a single continuous layer of cells resembling epithelium. This arrangement of cells when traced to the sides of the fibrinous mass shows them to be a direct continuation of the pleural epithelium (Plate II, Fig. 10). At the highest point of the mass the epithelial layer is flatter that at the sides, where the character of the pleural epithelium is preserved. In the crevices of the mass we find that this proliferation of the cells has produced several layers, but on the free surface the arrangement is that of a simple continuous layer. At one side of the preparation the proliferation is most remarkable. Projecting from the main mass of fibrin is a small band which touches the adjoining pleura with its extremity, leaving between its under-surface and the pleura a small free space. At the point of contact of this band with the pleura the epithelium has proliferated upon the under as well as the upper surface of the fibrin, so that counting from below outwards we have three layers of epithelium: first, pleural epithelium proper; second, a row of epithelium covering the under surface of the mass and continuous with the pleural epithelium of the same side; third, covering the outer surface a layer of cells continuous with the pleural epithelial layer extending beyond the point of contact. At the outer corner the fibrin has not fallen in direct contact with the pleura and we find the cells proliferated one over another for several layers perpendicular to the pleura until it reaches the fibrin, where it becomes a simple layer spreading out over the mass. Beneath the fibrin are several crevices left by irregularities in the mass. In these the pleural epithelium which remains intact has proliferated in such a way as to line them with a continuous layer of cells. Between these crevices, where the fibrin has come in direct contact with the pleura, no epithelium can be demonstrated and we find the connective tissue of the pleura widely proliferated into the fibrin. In one of the above described crevices we find the preserved pleural epithelium broken through by a single band of proliferating connective tissue extending into the fibrinous mass above, which, as in the other crevices, is covered with a single layer of epithelium (Plate II, Fig. 11). The elastica is continuous beneath the mass and we find no changes in the tissues underlying it.

Experiment IV.—Black rabbit. Introduced two pieces of fibrin into right pleural cavity.

Autopsy fourteen days after operation. Both pieces of fibrin organized to costal pleura, which with ribs are dissected out in one piece. Decalcified. Hardened in Müller-formalin. Microscopic examination. We find the fibrinous mass to be invaded by proliferated connective tissue, which has entirely organized the lower two-thirds. At the side of the main mass is to be seen an isolated glistening fibrinous clump (Plate II, Fig. 8). This mass, stained with picrocarmine, shows a high refractive index and brilliant yellow color. Its appearance is distinctly hyaline, and at its edges we find that the connective tissue has broken its way into the mass. No pleural epithelium can be seen beneath the fibrin. Blood-vessels can be demonstrated in the organized portion. Entire mass, as in Experiment III, covered with a layer of epithelium continuous with the pleural epithelium at both sides (Plate II, Fig. 9).

Experiment V.—Grey rabbit. Introduction of fibrin as in previous experiments.

Autopsy four weeks after operation. On costal pleura we find a mass somewhat smaller than the piece of fibrin introduced, and more flattened in appearance. Dissected out. Hardened in Müller-formalin.

Microscopic appearances. Mass largely converted into connective tissue. At base are to be seen here and there small bands of fibrin which appear hyaline in character. As we approach the surface these masses are more frequent and larger until we find only fine bands of connective tissue growing into the fibrinous mass proper. The entire mass covered with epithelium as in previous experiments. Elastica intact. Connective tissue beneath elastica unchanged.

Experiment VI.—The fibrin used in this experiment was produced by the action of a fibrin ferment on plasma obtained from horse's blood. The ferment was made after the method of Alexander Schmidt and Rauschenbach by expressing the contents of a fresh lymph gland and diluting it with water. By this method the plasma placed in a watch crystal formed in two minutes after the addition of the ferment a solid gelatinous clot. Microscopic examination of this clot showed it to be homogeneous in character. Careful examination of several specimens failed to show any hyaline-like masses.

Operation. Introduced into right pleural cavity of grey rabbit clot of fibrin immediately after coagulation.

Autopsy seven days after operation. Fibrin found organized to costal pleura near point of incision. Dissected out, hardened, embedded in paraffine.

Microscopic examination. At base of section part of intercostal muscle. Between muscle and fibrin connective tissue, which in deeper layers

runs parallel to muscle; in outermost portion it consists clearly of newly proliferated structure which penetrates into the fibrin. Isolated hyaline masses, giving reaction of fibrin with all staining methods, are to be found in the newly proliferated tissue. These masses are to be seen only where the organization is advanced. The fibrinous mass proper presents in part the homogeneous character described, and in part a fine network of fibres. Elastica intact except where broken through by incision, where we find newly proliferated tissue. No hyaline masses or fibrin found beneath elastica. The whole fibrinous mass covered by a single epithelial layer continuous with the pleural epithelium.

Experiment VII.—Grey rabbit. Small incision made between ribs on right side opening pleural cavity. Simultaneous injection through this incision of plasma and ferment, prepared as in previous experiment.

Autopsy in eight days. Fibrin organized to costal pleura. Hardened and embedded as usual.

Microscopic examination. Fibrin presents same appearance as in previous experiment. At base, organization surrounding hyaline-like masses. Through the entire mass we find the lymph cells from the ferment enclosed in the fibrin. In many places these cells and nuclei follow one direction, at the surface parallel to it, and in deep parts pressed into rows. Entire mass covered by a continuous layer of epithelium, beneath which are several layers of connective tissue. This organization of the fibrin on the surface can be traced from the sides where the proliferating tissue forms a continuation of the organization at the base. We find the connective tissue, in this zone beneath the epithelium, following a direction parallel to the surface, while the nuclei and leucocytes in the adjacent fibrin have the same parallel distribution. Plate II, Fig. 12, taken from this specimen, shows the epithelial covering, the organization beneath it, the leucocytes in the fibrinous mass, and the proliferating connective tissue extending upward from the base. At base of mass can be seen the remains of the incision where the elastica is broken. In sections where the incision does not appear the elastica is intact, with tissue below it free from fibrin and hyaline masses.

Experiment VIII.—Injection of 20 cc. of plasma into right pleural cavity of grey rabbit.

Autopsy one week later. Upon opening pleural cavity a flattened fibrinous mass, 18 by 12 mm. in surface dimensions, and 3 mm. in greatest thickness, is found. The same appears to be unattached. Hardened, embedded and cut. Microscopic examination of this fibrinous mass shows the same to be covered by a single layer of epithelium, except at

one point where the epithelium fails and the mass is the seat of a slight organization. This area forms about one-twentieth of the circumference and the organization extends into the mass for but one-fifteenth of its diameter. Fibrin homogeneous.

Further experiments merely confirmed the results already given and do not require special mention.

Summing up the results of these experiments, we find the following characteristics in all: Fibrin introduced as such, and fibrin-forming fluids when injected into serous cavities, become adherent to the serous membrane. The pleural epithelium remains intact under the fibrin until the process of organization begins. The epithelium of the pleura has the power to proliferate sufficiently to cover large areas (Experiment VIII). This extensive proliferation was found in all experiments after the sixth day. In one case (Experiment II), in which the autopsy was made forty-eight hours after introduction, the proliferation had not begun.

The proliferation of epithelium occurs before the organization by connective tissue. It is possible to find the epithelium preserved below the fibrin and its surface covered by an epithelial layer, the result of proliferation from the sides (Experiment III).

After the proliferation of epithelium over the mass, the connective tissue follows the same direction from the sides, forming a layer of connective tissue organization on the surface beneath the epithelium. The principal organization takes place at the base of the mass. This organization by connective tissue tends to separate masses from the main fibrinous deposit. These masses appear hyaline in character. They are often found lying parallel to the connective tissue fibres. Where cells or nuclei are injected with a fibrin-forming fluid, they become enclosed in the fibrinous mass and by the process of coagulation are often arranged in rows.

An application of these facts to the statements made by Neumann permits the following deductions: Neumann says that the epithelium covering the fibrinous exudate in his case was the pleural epithelium in its original anatomical position, and the fibrin was the result of degeneration of connective tissue lying beneath it. He excludes the

possibility of the fibrin being produced by exudation beneath the epithelium. That the epithelium could have been derived from and merely adherent to the opposite pleural layer he considers to be excluded by the presence of a pleural exudate. The explanation of Marchand that the epithelium was the result of proliferation (23) he sets aside with the statement that "in acute cases such a proliferation is inconceivable" (12).

In answer to Neumann's statement, and in support of Marchand's explanation, my experiments show an unfailing proliferation of epithelium on the surface in all cases in which the fibrin remained seven days in the serous cavity. The proliferation of epithelium in chronic cases, to which the observations of Buhl quoted by Neumann belong, has been observed by Riese (28), who found in tuberculous synovial cavities masses of fibrin covered by a layer of "endothelium" continuous with the "endothelial" layer of the sac. He distinctly states that he believes the cells to have proliferated over the mass. This observation agrees with those of Michon (26), who observed the same condition in synovial cavities. These cases are quoted by Neumann as examples of degeneration producing fibrin beneath an epithelial covering in its original anatomical relations.

Of still greater interest, in the light of these experiments, is the case quoted by Neumann (11), reported by Sultan (27), in which the fibrin lay between two layers of epithelium. Neumann believes that the superficial epithelial layer is the original epithelium, and that the epithelium marking the boundary between fibrin and connective tissue has been produced by regeneration. This case is, however, clearly one in which the original epithelium remained partly intact and the epithelium from the sides proliferated over the mass (compare Experiment III).

From these cases, experimental and others, we may safely conclude that the presence of epithelium overlying a fibrinous mass does not constitute proof that the fibrin was produced beneath it.

That the hyaline masses described by Neumann as characteristic of fibrinoid degeneration are in no way characteristic of such a process is shown by the presence of the same in all my experiments where

organization was advanced and where the process in no case extended beneath the elastica. In further proof of the same, I have in my possession a specimen of fresh thrombosis of the aorta in which in the middle of the thrombus mass, where no question of degeneration of connective tissue can be entertained, are to be seen hyaline bands and clumps easily distinguishable from the surrounding fibrin.

Finally, the arrangement of cells and nuclei in rows through the fibrin mass is not suggestive, as Neumann, Grawitz and Schleiffarth would have it, that the same are remaining connective tissue nuclei and owe their arrangement to this fact.

The older theory that the cells found in fibrinous exudates are leucocytes enclosed in the fibrin is in no way impaired by this observation of Neumann, Grawitz and Schleiffarth. On the contrary, this arrangement tends to show that the cells were present when the fibrin was in a fluid condition and that they owe their arrangement to the process of coagulation.

TUBERCULOSIS.

The microscopic appearances found in the fine, closely adherent fibrinous masses on the pleura covering the affected portions of tuberculous lungs are so different from those which characterize the cases just described that they may well be considered separately.

Here we must at once allow that the characteristic appearance described by Neumann does to a large extent suggest a degeneration of the connective tissue and the production directly from the same of fibrin. So intimately adherent are the fine bands of fibrin to the connective tissue, and so diversely distributed between its fibres, that we are led at once to the belief that here we are dealing with a different process. It is only when we return to the elastica that the necessary evidence it wanting.

It was the lack of this evidence which led Professor Orth, in his address at Frankfort, based on the specimens of Vassmer and myself, to state that "no definite position can be taken on this question until the fibrinous masses can be demonstrated under the elastica." Ziegler (30) describes the condition found in such cases as hyaline degeneration of connective tissue similar to amyloid degeneration, and

does not agree with Neumann's explanation of the same. He regards the change as one of degeneration.

As this investigation has been concerned principally with that portion of the question dealing with acute non-specific processes, my experience with the tuberculous cases has not been sufficient to base an opinion on negative evidence. In no case have I been able to demonstrate the masses beneath the elastica. So different are the microscopic appearances in these cases from those found in the acute processes already presented that I add for comparison three photo-micrographs from a case of tuberculous pleurisy. We see here the masses and bands described by Neumann, which are by their distribution suggestive of a direct degeneration of connective tissue into fibrin.

Plate I, Fig. 5, shows a section through the pleura stained by Weigert's method. We see the masses of fibrin covering the surface and extending into the connective tissue. The connective tissue is infiltrated with cells and shows cross sections of blood-vessels.

Plate I, Fig. 6, shows a section of the same pleura with the elastica stained. Here we see that the masses lie above the elastica, and that in fact the amount of connective tissue described by Vassmer as normal lies undegenerated above the elastic stripe. Therefore all connective tissue above this undegenerated zone must at one time have been produced by proliferation, and if the fibrin is the result of degeneration, it is degeneration of a new tissue alone and not of the normal structures of the pleura.

Plate II, Fig. 7, shows the masses at the surface of the pleura and the oval nuclei described by Neumann, which he believes to be the free nuclei of the degenerated connective tissue.

Such cases can be explained, as Marchand (23) explains them, as merely the irregular organization of a previous exudate which isolates masses of fibrin.

The parallel direction of the bands of fibrin and their relation to the connective tissue is not suggestive of the picture seen in the organization of exudates, in which the young connective tissue often grows perpendicular to the pleura and seldom shows such a regular arrangement of its fibres. We need only the confirming fibrin beneath the

elastica to solve the problem and separate these cases from the simple exudative form of pleurisy to which they bear but a very slight resemblance. Until the supporters of the theory of fibrinoid degeneration can establish this fact the question of the true character of tuberculous cases will remain in doubt.

In closing I wish to take the opportunity to express my thanks to Professor Orth for the material which he has placed at my disposal, and for the kind advice and support he has given me at all times during this investigation.

DESCRIPTION OF PLATES I AND II.

PLATE I.

All of the figures are photo-micrographs. Objectives of the Winkel-Flourit system were used.

Fig. 1, × 150. Objective, 4.5 mm. Ocular No. 2. Stain, Van Gieson, orcein. Section of pleura covering consolidated lung from case of pneumonia.

At *a*—*a'*, pleural epithelium intact. Here and there single, detached epithelial cells adherent to fibrin *c*. At *B*, elastica stained with orcein. Between *a* and *B*, hæmorrhagic infiltration beneath epithelium.

Fig. 2, × 110. Obj. 4 mm. Oc. No. 1. Stain, hæmalum, orcein. Section from case of purulent pericarditis.

At *c*, thick fibrinous exudate containing leucocytes. *a*—*a'*, continuous pericardial epithelium. *B*, elastica stained with orcein. Between *a* and *B*, layer of healthy connective tissue. *D*, heart muscle, cross section.

Fig. 3, × 110. Obj. 4 mm. Oc. No. 1. Stain, hæmalum, orcein. Section of pleura covering hæmorrhagic infarct of lung.

c—*c'*, fibrinous exudate containing leucocytes. *a'*—*a'*, continuous pleural opithelium. *B*, elastica stained with orcein. Between *a'* and *B*, layer of healthy connective tissue. *D*, hæmorrhagic infarct of lung.

Fig. 4, × 110. Obj. 4 mm. Oc. No. 1. Stain, picrocarmine, orcein. Section of adherent pleural surface, between adjacent lobes of lung from case of pleurisy.

c—*c'*, point at which adjacent surfaces are adherent and in which can be seen fibrin mass at *a*. *A*—*A'*, remains of pleural epithelium of upper surface. At *a'*, cells of lower surface below fibrin mass. At *B* and *B'*, elastica stained with orcein. Between *B* and *c*, healthy connective tissue.

Fig. 5, × 60. Obj. 8 mm. Oc. No. 1. Stain, hæmalum, Weigert fibrin stain. Section of pleural surface covering tuberculous lung.

A, fine fibrinous bands running parallel with the surface. *c*, fibrin in blood-vessel. *f* and *f'*, fibrinous exudate in alveoli of lung.

Fig. 6, × 110. Obj. 4 mm. Oc. No. 1. Section of pleura from same case as Fig. 5. Stain, Van Gieson, orcein.

a, fibrinous mass covering the surface; *a'*, isolated bands of fibrin. *B*, elastica stained with orcein. *c*, connective tissue above elastica. *f*, deep layer of serosa.

PLATE II.

Fig. 7, × 110. Obj. 4 mm. Oc. 1. Section through pleura. Same case as in Figs. 5 and 6.

a, fibrinous masses covering surface of pleura. *a'*, single fibrinous band separated from mass by connective tissue. Fibrinous mass and surrounding tissue infiltrated with cells, many of which have oval nuclei. At *B*, distended blood-vessels.

Fig. 8, × 30. Obj. 16 mm. Oc. 1. Stain, picrocarmine.

Section of fibrin in organization from Experiment IV. *a*—*a'*, connective tissue surrounding glistening hyaline clump at *B*.

Fig. 9, × 60. Obj. 9 mm. Oc. 1. Stain, hæmalum. Section of fibrin in organization from Experiment IV.

a—*a'*, epithelium covering fibrin. *c*—*c'*, fibrin consisting of hyaline masses and clumps. *B*—*B'*, organization at base of fibrin. *D*, connective tissue of serosa.

Fig. 10, × 110. Obj. 4 mm. Oc. 1. Stain, hæmalum. Section of organized fibrin from pleural cavity of rabbit. Experiment III.

C, fibrin organized to pleura. *a*, pleural epithelium continuous with layer of epithelium *B*, covering fibrin. *D*, intercostal muscle.

Fig. 11, × 160. Obj. 3 mm. Oc. 1. Stain, alumcarmine. Section at base of organized fibrin from pleural cavity of rabbit. Experiment III.

a—*a'*, pleural epithelium broken through by band of connective tissue *B*. *c*, proliferated epithelial cells on under surface of fibrin *D*. Between *a'* and *c*, crevice beneath mass. *f*, connective tissue of pleura.

Fig. 12, × 70. Obj. 7.5 mm. Oc. 1. Stain, hæmalum. Section of fibrin mass from Experiment VII.

a—*a'*, single continuous layer of epithelium covering fibrin mass *c*—*c'*. *D*, connective tissue organizing fibrinous mass from beneath. *c*—*c'*, connective tissue organizing mass from surface.

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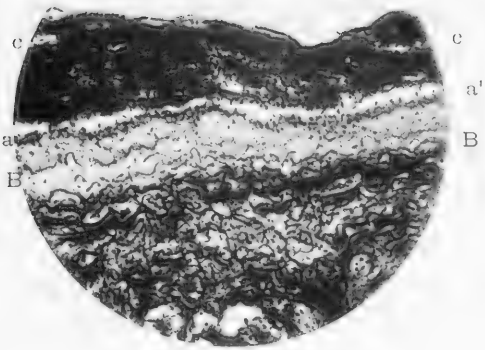

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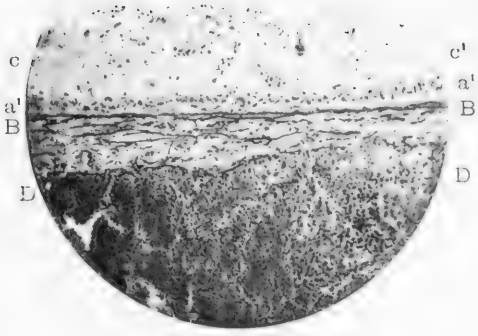

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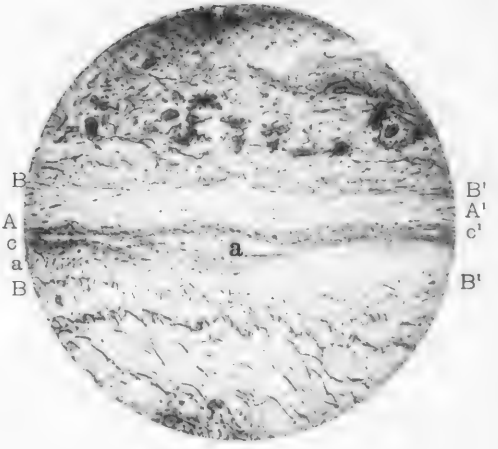

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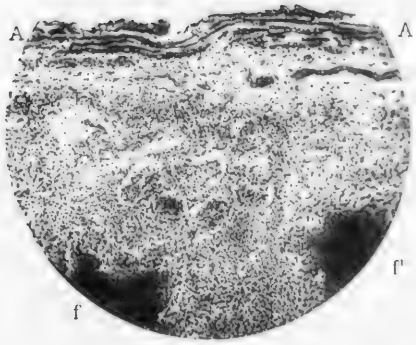

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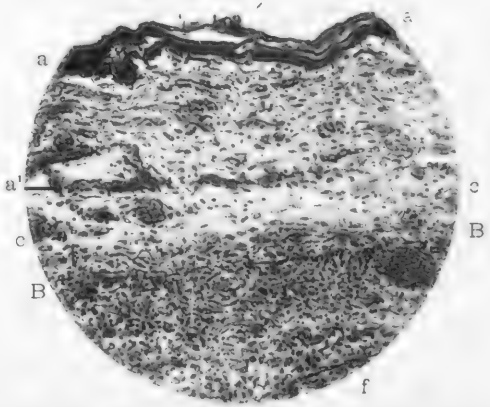

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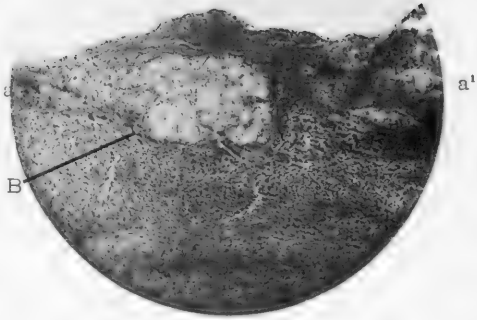

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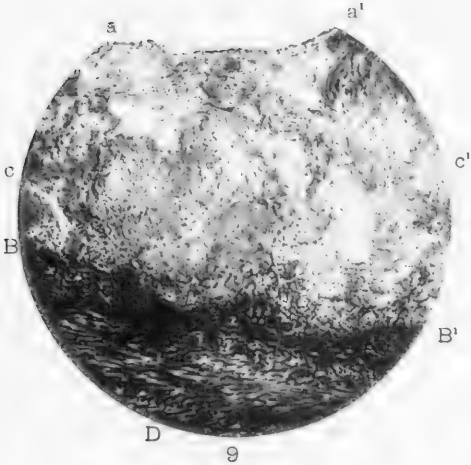

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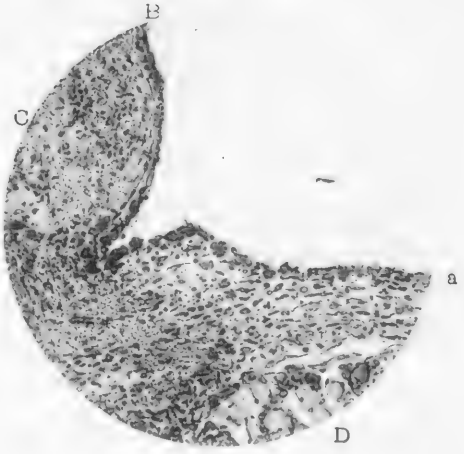

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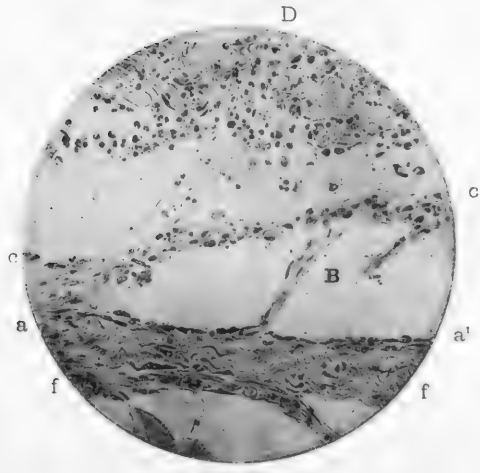

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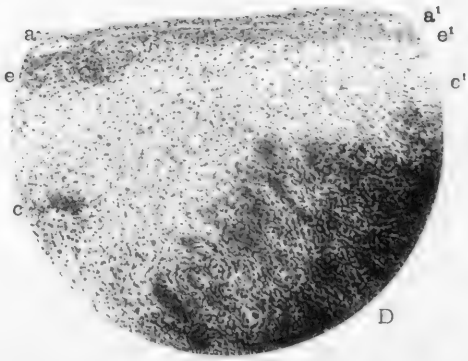

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THE FATE OF THE GIANT CELLS IN HEALING TUBERCULOUS TISSUE, AS OBSERVED IN A CASE OF HEALING TUBERCULOUS MENINGITIS.

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Plate III.

The investigations into the nature and the importance of the giant cells of tuberculous tissue have not led to uniform results. The views differ more particularly with respect to the genesis and to the vitality of these peculiar elements.

While Rokitansky,* Virchow and others had observed, and in part described, the giant cell, it remained for Langhans, in 1868, to first give a full and satisfactory account of these cells, and to advance the two most prominent hypotheses in regard to their origin; namely, that they are the product of one cell, or formed by the fusion of many cells. The investigations of Langhans were soon followed by a very large number of studies concerning the giant cells of tuberculous and of other inflammatory, as well as of resorptive, processes. The literature upon the mode of formation and the nature of giant cells is very extensive, and it would not materially advance the object of this article to review it completely. I would, however, refer to the studies of Schüppel, Köster, Heidenhain, Weiss, Ziegler, Brodowsky, Marchand, Ranvier, Baumgarten, Cornil, Malassez, Martin, Arnold, Kiener, Weigert, Metchnikoff and his students, Taber, Kostenich and Wolkow, Krückmann, Welcker, and of many others, as indicating the amount of work bestowed upon the problems connected with multinucleated giant cells. The majority of these investigators review the pre-existing literature more or less thoroughly. Thus Marchand, in 1883, in his article on the giant cells that occur in connection with the absorption of foreign bodies, refers to no less than forty-five previous publications bearing on the subject of giant cells in general.

Koch's discovery of the tubercle bacillus, and the further demonstra-

* The references to the literature are arranged alphabetically at the end of the article.

tion of the important relations of this bacillus to the giant cell, gave rise to continued vigorous investigation and speculation concerning the Langhans cell. While the views of the authors in regard to the genesis of the giant cell of tuberculosis are so divergent and the possibility of different modes of development so marked as to indicate that, in all likelihood, the giant cells may indeed be the outcome of more than one process, the opinions concerning the vitality and the importance of this element have differed in a more precise and distinct manner, so that the investigators may be separated into two quite well defined schools, namely, those who, headed by Baumgarten and Weigert, regard the cell as an element on the way to destruction, as necrobiotic from its very inception; and, on the other hand, those who, led by Metchnikoff, are convinced that the giant cells of tuberculosis are living, active and defensive (mesodermal) cells.

Baumgarten, in his classical study of the histology of tubercle, concludes that the giant cell is the product of multipolar nuclear division in a single mesodermal or epithelial cell, the body of which fails to divide on account of degeneration in its interior, due to the necrobiotic action of the bacilli that it usually harbors. Weigert agrees with Baumgarten and has vigorously opposed the contradictory claims of Metchnikoff and his followers.

In his original communication concerning the bacillus of tuberculosis, Koch is inclined to regard the giant cells as somewhat permanent elements possessing more vitality than the bacilli they usually contain, and holds that the bacilli are able to persist in the giant cell simply because one generation succeeds another. He also points out that degenerate bacillary forms are not infrequently met with in the interior of giant cells. Later Koch seems to favor more directly Weigert's opinion. Metchnikoff has persistently championed the view that the giant cells are defensive, mesodermal phagocytes, produced in various ways, at times by direct nuclear division, at times by indirect segmentation of the nucleus, and still at other times by the fusion of epithelioid mesodermal cells without special nuclear changes. In one of his first articles on this subject he details the histological changes produced by the bacillus of avian tuberculosis when injected into the small rodent, Spermophilus guttatus, to which our gopher is related. He shows how the bacilli are taken up by the numerous giant cells that come into existence, and in the interior of which they undergo marked changes, both as to staining and to form, which must be regarded as clearly retrogressive in character. He furthermore demonstrates that giant cells may multiply by simple fission without mitosis, and also describes appearances that indicate that two or more giant cells may coalesce into a larger cell complexus.

Ssudakewitsch brought further proof of the activity of giant cells by showing that the giant cells found in the skin in an Eastern disease, pascha-churda, and in lupus, are capable of digesting elastic fibres.

Arnold, to whom we owe extended studies upon the manner of formation of, and the nuclear changes in, giant cells, was at one time inclined to divide giant cells into progressive and retrogressive forms, and to designate those of tumors as belonging to the former, and those of tuberculous tissue to the latter class; but after he had been able to demonstrate that even the tuberculous giant cells have the power of further development he no longer regarded any such distinction as justifiable. The fact that giant cells are destroyed in tuberculous tissue does not in any way invalidate this statement, because this fate they suffer in common with all the cells of this tissue—the point being that the giant cells do not have in them the seed of retrogressive metamorphosis from their very origin.

Welcker, not long ago, repeated the original experiments of Metchnikoff with avian tuberculosis upon Spermophilus guttatus. He did not find any evidences of multiple karyokinesis in the epithelioid cells and questions this mode of formation for giant cells. He regards direct nuclear division as the most frequent mode of formation, but does not exclude fusion. He could not demonstrate the amœboid movement of the giant cells which was claimed for them by Metchnikoff. He found in the interior of the giant cells peculiar sausage-shaped masses that he demonstrated to contain iron, and which he regards as bacilli changed by cellular activity.

The concentric, lamellated, calcareous concretions, described by Schüppel in tuberculous lymph nodes, and also briefly referred to by Ziegler in the seventh edition of his work on Pathological Anatomy (page 97), are regarded by Metchnikoff as due to the calcification of tubercle bacilli in the interior of cells. According to Metchnikoff the process of calcification can be well traced in the Algerian rat, an animal relatively insusceptible to tuberculosis, in which the tuberculous tissue, even many months after the experimental inoculation, consists of living epithelioid and giant cells, the latter enclosing dead bacilli in the form of the characteristic earthy bodies. He regards it as very probable that the phosphate of lime is laid down by the giant cell itself in the reaction against the bacillus. The final fate of the giant cells in this form of tuberculosis is not discussed by him.

The researches of recent years, carried out by Metchnikoff and his students at the Pasteur Institute in Paris, all warrant, according to their authors, conceptions quite similar to those first advanced by Metchnikoff

concerning the giant cells, and the general conclusion of this school is that it is of essential importance in the fight against tubercle bacilli that large protoplasmic masses with many nuclei are formed, so that the activity of the intracellular processes may be heightened because the bodies to be digested are very resistant. This would not appear to be at all a strange process when we remember that plasmodia are observed so generally in connection with cellular digestion in the lower as well as in the higher animals.

The modes of formation of giant cells, the many possible sources of their origin, their appearances, their relation to the bacillus of tuberculosis and to other parasites, as well as to foreign bodies, the degenerative changes that may occur in their interior, and many other interesting and important features have all been carefully and repeatedly studied. But the further definitive changes that may occur in these elements when the tubercle bacilli or other micro-organisms die or are absorbed before the cells have undergone necrosis, or after the foreign substances have been completely removed, have, strange to say, received but scant attention. Tuberculosis not infrequently heals, syphilis heals, actinomycosis heals, and foreign bodies are often completely absorbed. Are the giant cells, so generally present in these conditions and which sometimes persist even after the active cause that brought them into existence has been removed, capable of any progressive changes? Do they continue to exist as giant cells, or does complete healing or absorption mean their removal by way of disintegration? Manifestly a decisive solution of this rather neglected problem, in connection with the giant cells of tuberculous tissue, for instance, would materially aid us in arriving at some definite conclusion concerning their nature, whether they are to be regarded as purely necrobiotic elements, or as structures possessing an independent vitality that may outlast that of the tubercle bacillus, and in the destruction of which they perhaps play an active rôle.

The exact histological details of healing non-degenerated tuberculous tissue have been lost sight of on account of the general impression that the healing is accomplished, in the main, through necrosis and necrobiosis of the tuberculous tissue, together with calcification,

followed by more or less regeneration of the specific tissue elements, the principal part of the resulting defect being bridged over by cicatricial tissue.

That tubercle does not always undergo caseation was noted by Cruveilhier (quoted by Straus), who states that tubercles of the lungs, in particular, are curable at all periods of their development by means of a sort of fibroid encystment (tubercules de guérison). Again, Grancher states that all tuberculous granulations that develop slowly become fibrous and heal, that is, they are transformed into a sclerotic and inoffensive anatomical product in the form of a "fibro-caseous neoplasia."

D. J. Hamilton, in his Text-Book of Pathology, says that sometimes the tubercle does not caseate, but continues to live on and to develop into what seems to be its ultimate stage of organization. In or quite near to the centre is usually a giant cell, around which there may be found smaller giant cells. From the periphery of the giant cell processes come off that by subdivision produce a reticulum which is more condensed at the margins of the tubercle. In the meshes lie lymphoid corpuscles and one or more giant cells. This is the reticular tubercle first described by Wagner. As this tubercle becomes older the reticular giant-cell system is replaced by a simple mass of fibrous tissue; the giant cell becomes more and more fibrous at its periphery, and the protoplasm of the cell becomes transformed into or secretes the fibrous margin, which is covered with nuclei that appear to be derived from the giant cell. During this process of fibrous transformation the bacilli have vanished.

The investigators that have studied the action of dead tubercle bacilli upon the tissues make no statements as to the ultimate fate that befalls the giant cells that may form in the cell accumulations around the dead bacilli (Straus).

The precise manner of healing of tuberculous peritonitis after laparotomy has been made the subject of a considerable number of investigations, the results of which differ somewhat, the variations depending, it would seem, upon the stage of the process at which the operation was performed. Osler reports the results of the microscopic examination in the case of a woman who died of acute pneumonia 4½ months after a laparotomy which had been followed by marked improvement of the abdominal condition; the peritoneal tubercles were changed to fibrous masses containing giant cells with fatty granules, abundant bacilli, but no caseous material. Pichini, Bumm, Riva and D'Urso found that in healing tuberculous peritonitis after laparotomy (in man) the tubercles are replaced by fibrous tissue, and, according to Bumm and D'Urso, the

giant cells are destroyed by wandering cells. Among those who have studied the question experimentally may be mentioned Kichensky and Stchégoleff, both of whom regard the improvement and healing that may follow laparotomy in experimental tuberculous peritonitis in guinea-pigs, dogs and rabbits as due essentially to phagocytosis, to infiltration with round cells, to the formation of fibrous tissue while the "specific elements of the tubercle" disintegrate and undergo absorption. Mazzoni, Nannotti and Baciocchi, and especially Gatti, emphasize dropsical degeneration of the epithelioid cells as an essential step in the healing. Gatti, who studied this problem in guinea-pigs and rabbits, but principally in dogs because of their greater resistance and of the more marked chronicity of the tuberculosis, comes to the conclusion that the perfect healing, macroscopic, histological, and biological, that unquestionably does follow simple opening of the peritoneal cavity, when the tubercles of the peritoneum have reached a certain stage—the fibroid but not the caseous—does not depend upon inflammatory reaction nor upon proliferation of the connective tissue, but upon a dropsical degeneration of the epithelioid cells, which melt together to form small spaces, while the nuclei are dissolved, and finally the remnants of these cells disappear completely, together with the lymphoid cells, leaving simply the preexisting stroma; fibroblasts and karyokinetic figures do not occur. Gatti reasons that the poison that kills the epithelioid cells does not attract leucocytes; that the bloody serum, which forms after the laparotomy, kills the bacilli or so weakens them that the resulting proteins cause slow degeneration of the cells; and that, therefore, the prime factor in the operative healing of the peritoneal tuberculosis is the exudation of serous fluid into the abdominal cavity. When the tubercles have become caseous laparotomy may arrest further casecation, but has no direct influence upon the removal of caseous masses.

It will have been observed that practically all of the authors quoted fail to record any observations as to the exact changes in the giant cells in healing tuberculous peritonitis—Gatti rarely mentions the giant cells in his articles—and, furthermore, that none of the investigators attribute any active part in the healing process to the cells of the tuberculous tissue itself, all speaking solely of degeneration, disintegration and absorption of the cells of the tubercles.

In his work, "Die causale Behandlung der Tuberculose," Klebs devotes a chapter to the consideration of the histology of the healing tubercle, as observed in guinea-pigs suffering from experimental tuberculosis and treated more or less successfully with the products of the tubercle bacillus. He advances the view that the proliferation caused by the

tubercle bacillus protects the bacillus against the bactericidal action of the fluids of the tissues, hence the latency of many local tuberculous infections. When the tuberculous tissue loses its protective properties on account of degenerative changes, the bacilli may die or they may multiply, depending upon various local and general conditions. Under the influence of the tuberculin and its purified modification, tuberculocidin, there is produced an exudation which deprives the cells of the tuberculous infiltration of their protective powers and the bacilli are killed by the serum. Now, the cells of tuberculous proliferations do not possess unlimited powers of proliferation, and hence tuberculous tissue is susceptible of healing. After the death of the bacilli, the tuberculous tissue may disappear partly by necrosis, regeneration and cicatrization, partly by transformation into mature tissue. While the capabilities of many of the cells are exhausted, so that they are merely useless elements that are best removed, tuberculous tissue may yet contain various cells of connective tissue as well as epithelial origin that possess an independent vitality and outlive the tubercle bacilli. Such cells may, in the course of further events, become component parts of apparently quite normal structures. Thus the giant cells in the alveoli of healing pulmonary tuberculous areas may subdivide into small uninuclear cells; the nuclei become richer in chromatin, karyokinetic figures form, and the cells apply themselves to the alveolar walls and become part and parcel of its lining. The cells that do not form, or are not needed to assist in the formation of the alveolar lining, are thrown out. In the healing of tubercles in the periportal tissue of the liver, the giant cells which, according to Klebs, are formed from the lining of the bile ducts, separate again into individual cells. In healing splenic tubercles of guinea-pigs the giant cells, which are all intravascular, become reduced in size by contraction of the protoplasm and remain for a long time after the disappearance of the bacilli, but Klebs thinks that they are finally divided up into endothelial cells. The giant cells in tubercles in the lymph nodes are formed by the endothelial cells of the lymph vessels, and may, after the destruction of the tubercle bacilli, separate and revert to endothelial cells again; such giant cells, therefore, are closely related to the intravascular giant cells.

Klebs regards the non-epithelial giant cells in connective tissue as coming from the angioblasts. He observed in a case of recurrent tuberculous pericarditis that the miliary tubercles were surrounded by wide vessels which passed into the tuberculous tissue, where they disappeared, their place, however, being taken by very large giant cells which were surrounded by round cells. The protoplasm and the processes of the

giant cells stained more deeply with the ponceau dye than the surrounding cells. The nuclei were arranged with their long axes vertical to the surface and usually around a clear centre. While not able to trace a distinct and immediate connection between the giant cells and the vessels, Klebs seems to believe that as the vessels are compressed by granulation tissue they may fall into fragments or pieces that remain filled with various substances, that the endothelial cells multiply and the angioblasts form giant cells which may subsequently divide into endothelial cells, although the only evidence he has seen of this latter change was not found in human tuberculosis, but in the tuberculous spleen of guinea-pigs.

While studying the vascular changes in the leptomeninx in a case of clinically anomalous tuberculous leptomeningitis of an unusually long duration, my attention was directed to the relatively excessive number of giant cells present and to certain peculiar changes presented by them, that seemed to point quite clearly to the occurrence of other than merely retrogressive processes in the specific proliferations. I consequently utilized the opportunity to study more particularly the giant cells of this form of healing meningitis. The results of this part of my work are embodied in this article; further observations made in connection with suitable experiments are reserved for publication later.

Tuberculous leptomeningitis may, like tuberculous inflammations of other serous membranes, heal spontaneously. Dujardin-Beaumetz, Bauer, Sejournet, Weir, Nilsson, Martel, Klein, Freyhan, as well as many others, describe cases diagnosed clinically as tuberculous meningitis which ended in recovery. Ord describes a case diagnosed as cerebral tuberculous meningitis in which recovery followed after drainage through a trephine opening into the subdural space.

While the diagnosis in such cases as the above may be open to criticism, Fütterer examined post mortem a patient who had suffered two years before death from a disease diagnosed by Leube as spinal tuberculous meningitis, and found the spinal leptomeninx to contain shrunken, calcareous, miliary tubercles, *i. e.* he furnished the anatomical proof of a healed degenerating tuberculous leptomeningitis.

Atypical, subacute, and chronic, sometimes latent, forms of tuber-

culous meningitis also occur. Whereas, according to Strümpel, the average duration is between three days to one and one-half weeks, Seitz mentions two cases of forty-two and fifty-four days, Ludwig Meyer one of forty-nine days, Neudörffer one of fifty-six days, and Busse one which lasted at least sixty days. These cases occurred in adults.

In the case described by Busse, which occurred in a woman thirty-seven years old, the pia was adherent to the cortex and contained numerous flattened and hard elevations of irregular size and outline. In the Sylvian fissures were small, grey, translucent nodules. Caseation was absent. The areas of thickening consisted of interlacing bundles of fibrous tissue, containing here and there masses of cells and also occasional giant cells. Tubercle bacilli were found in small numbers inside as well as outside the giant cells. Evidently there was here a chronic tuberculous inflammation in process of arrest and healing.

The present case was that of a man, white, aged 25, admitted into the service of Dr. Norden, at the Cook County Hospital, December 13, 1895, with a history of having been sick for 35 days before admission with headache, some nausea and pain in the stomach. He had been working in a rolling mill before he became sick. He had never had any serious illness before and denied all venereal infection. Precise information could not be obtained because he was stupid and dazed.

Physical examination showed a medium-sized, rather slender and poorly nourished man; the organs of the chest and abdomen were quite normal. The tongue was dry and coated. There were some spots on the arms that were thought to be rather suspicious of syphilis. The cervical glands were enlarged. The temperature, pulse and respiration were normal at the time of admission. He was placed upon iodide of potassium, one gramme four times daily.

During the days succeeding his admission he complained at times of very severe headache, more so at night, and would clutch his head with his hands and moan. There were no focal cerebral symptoms. After a week or so the potassium iodide was increased to two grammes four times daily, and further notes of headache were not made. The record shows that while his pulse and respiration remained normal the temperature would occasionally mount up to 101° F. There was marked costiveness and much nausea, the appetite being poor. After December

30th his temperature but rarely rose above 98.5°. Examination of the urine was negative except for hyaline casts. Examination of the eyes with the ophthalmoscope on February 15, 1896, by Dr. Wescott, showed the disk to be poorly defined on the right side, less so on the left. No tubercles were found in the iris.

The emaciation increased somewhat; the symptoms and signs of pneumonia appeared shortly before his death on March 6, about four months after the beginning of his sickness.

The clinical diagnosis was cerebral gumma and chronic nephritis.

Post-mortem examination.—Ten hours after death.

Anatomical diagnosis.—Chronic tuberculous leptomeningitis; chronic tuberculosis of the peribronchial lymphatic glands; acute bronchitis; lobar pneumonia; adhesive pleuritis; adhesive perihepatitis, perisplenitis and periappendicitis; chronic gastritis; parenchymatous nephritis; venous angioma of the liver; decubitus.

The body is poorly nourished and of rather slender build. The rigor mortis is marked. There is a small bed-sore over the sacrum.

The peritoneal cavity is empty; there are adhesions between the liver and the diaphragm and between the gall bladder, the duodenum, and the colon, also around the spleen and around the vermiform appendix.

The pleural cavities are empty; adhesions are present posteriorly over both lungs. The pharynx, larynx and trachea are normal. The parenchyma of the lungs is spongy and pale grey in color, except the lower right lobe, which is solid, and rather homogeneous; from its cut surface, which is of a red color, small whitish casts are scraped away. The bronchi contain muco-pus. The peribronchial lymphatic glands are enlarged, containing caseous areas, calcareous masses and small yellowish nodules.

The pericardium is empty and the surface of its layers is smooth. The heart weighs 240 grammes, the endocardium is smooth, the muscle uniformly firm and greyish brown in color. The aorta is smooth.

The spleen weighs 200 grammes and on section is seen to contain much fibrous tissue.

The kidneys weigh 230 grammes together; the capsules are free, the cortical markings distinct, the medullary rays being especially plain, the consistence firm. The ureters, seminal vesicles, and prostate are normal.

The liver weighs 1400 grammes; of firm consistence; near the anterior surface of the left lobe is a venous angioma about 2 cm. in its longest diameter. The gall bladder is normal.

The œsophagus is normal. The mucous membrane of the stomach is thickened and red. The large and small intestines are normal. The pancreas is normal.

The scalp, the skull and the dura are normal. At the base of the brain there is much turbid serous fluid and a thick layer of rather firm, translucent, gelatinoid material matting the structures together quite firmly. Along the arteries in both Sylvian fissures are numerous whitish and greyish yellow nodules, of pin-head size and larger, imbedded in the translucent material with which they seem to be infiltrated. The ventricles are dilated, containing much turbid fluid, the lining being rather soft. The brain substance is normal.

Both ears are normal; the sphenoidal sinuses are normal. The spinal cord was not examined.

Bacteriological examination.—The lungs were found to contain, in the pneumonic lobe, the Micrococcus lanceolatus, which was present in cover-glasses prepared from the exudate and was also isolated in the pure cultures.

The liver contained a coccus corresponding to the staphylococcus pyogenes albus, but cultures made from the spleen and the kidneys remained sterile.

Cover-glasses prepared from the meningeal exudate did not contain any micro-organisms when stained with ordinary dyes, and special stains for tubercle bacilli failed to reveal any bacilli (4 cover-glasses). A glycerine-agar tube inoculated with a liberal quantity of meningeal exudate remained sterile after having been in the thermostat for four days; at the end of this time it was sealed and returned to the thermostat, and on May 26, 1896, it was found that a pure growth of tubercle bacilli (mammalian) had developed in typical form.

Histological examination.—There is marked degeneration and desquamation of the epithelial cells lining the convoluted tubules of the renal cortex.

The changes in the leptomeninx and its vessels presented many features of interest and they were, therefore, extensively studied in serial sections prepared in the following manner: The tissue had been fixed and hardened in nearly absolute alcohol; small pieces from various parts of the basal leptomeninx, usually cut so as to have one or two vessels running through the centre of the longer diameter, were cleared in cedar oil and imbedded in paraffin; extensive ribbons of sections at right angles to the vessels were made and fastened on the slide with albumin fixative; the different series of sections would vary in thickness; some were 4μ, others 6μ, others 10μ, depending upon the exact minimal thickness at which perfect series could be obtained. The majority of the slides were stained with Delafield's hæmatoxylin; differentiated with 1 per cent. aqueous HCl solution, washed carefully and for a long time in water,

dehydrated in absolute alcohol, cleared and counterstained with oil of cloves containing in solution enough eosin to give it a rich red color, the excess of the oil being removed by xylol. Van Gieson's hæmatoxylin and picric-acid-fuchsin method was also used, and a number of slides set aside for the staining for tubercle bacilli were either colored with hæmatoxylin, stained with carbolfuchsin and dehydrated with xylol-aniline oil after a short bath in the iodine-iodide-of-potassium solution, or stained directly with carbolfuchsin, decolorized with 20 per cent. sulphuric acid and counterstained with methylene blue.

The inability to obtain the ponceau dye, used and recommended by Prof. Klebs for the purpose of counterstaining sections of healing tuberculous tissue, prevented its employment.

The changes were now studied in the various series of sections which were found to include in a perfect manner very many larger and smaller miliary tubercles; the appearances of the giant cells could be easily followed through the whole thickness and extent of the cell.

The vascular changes.—The intima of the larger arteries at the base of the brain may show a more or less well-marked thickening, due to the presence of a sparsely nucleated fibrous tissue situated between the lumen of the vessel and the tunica elastica. This thickening is best marked where the adventitial and extravascular changes to be described are most intense. In many instances this intimal thickening contains districts of hyaline degeneration which assume a faint bluish-violet tinge when stained with hæmatoxylin and eosin. The thickening in the larger arteries is usually semicircular in its extent and tapers from the thicker central part towards the ends of the semicircle, the elastic layer below it being intact and the endothelial lining quite distinct.

The smaller arteries further out in the Sylvian fissures also exhibit, more especially at points corresponding to the larger adventitial foci, quite extensive chronic fibrous thickening of the intima that at times greatly narrows the lumen of the vessel. In some of the series of the sections the deeper parts of the subendothelial fibrous tissue are the seat of advanced typical hyaline degeneration, but distinct caseation is not present. In other places the parts near the muscular coat are quite rich in cells, the amount of fibrillation being much less than in the strata nearer the endothelial lining, so that here the tissue is composed of round or oval cells with quite deeply stained nuclei, lying in a very loosely meshed stroma. In one instance an indistinct giant-cell-like body that is recognized through three sections, each 5μ thick, is found lying among the fibroblasts (Fig. 5, Plate III); this cell is small and seems to consist of a mass of very closely aggregated nuclei with a minimum amount of

cell body. In some places, again, the deeper parts of the proliferation contain spaces without any recognizable endothelial lining, but nevertheless filled with blood.

The endothelial lining over the thickenings is quite distinct and usually composed of small, closely set, rather typical cells. The cells in the layers immediately under the endothelium are not distinguishable, so far as shape and size are concerned, from the cells of the endothelial lining proper.

The elastic layer is everywhere intact, but the connections between it and the intimal thickening are apparently so loose and free that the thickened intima has often become more or less detached.

The extravascular changes.—The translucent material mentioned in the macroscopic description as surrounding the basilar vessels and other structures is composed, generally speaking, of a loosely arranged, richly cellular, very young fibrous connective tissue, throughout which are scattered a remarkably large number of variously shaped giant cells, often enclosed in small nodules of an indistinct concentric arrangement that might be considered as changed miliary tubercles. Caseation is almost absent; only in one or two places are small districts of caseation found in the adventitial foci of smaller arteries, and the caseous material seems to be enclosed in a capsule of more dense though quite cellular connective tissue. The adventitia of the smaller arteries in the Sylvian fissure and elsewhere present very small nodal enlargements made up of similar young fibrous tissue which also contains very many giant cells.

Many of the cranial nerves are the seat of interstitial changes similar to those above outlined.

The tissue referred to as young fibrous connective tissue may present a plainly fibrillated, sometimes lamellated, more often perhaps a very loosely arranged indistinctly fibrillated, granular or homogeneous matrix. Plain fibrillation, with occasional lamellation, is found at the periphery of the adventitial foci of the arteries, and this tissue is not so richly cellular; in such areas the endovascular thickening may be very pronounced. Mostly, however, this tissue consists of a matrix that stains more or less deeply reddish with eosin, being often composed of loosely and irregularly arranged bands of a fibrillar or granular structure of varying width and density; or the matrix may be quite homogeneous.

In this matrix lie various kinds of cells; usually the cells are scattered, but occasionally the cell infiltration is so marked that the intercellular substance can hardly be made out. In addition to the giant cells already mentioned, two other kinds of cells are distinguished: (1) Cells with rather large, lightly staining, occasionally almost vesicular

nuclei, that are oval, oblong, spindle-shaped, or of long drawn-out and irregular forms. The cytoplasm of these cells often blends with the intercellular substance so that the exact limits or shape of the cell body cannot always be made out; usually the cell body corresponds somewhat in shape with that of the nucleus; it is finely granular, almost homogeneous and stains faintly with eosin. (2) Cells, small in size, with relatively large, round or oval, very deeply staining nuclei surrounded by a small rim of reddish cytoplasm; these cells, which are the most numerous, would correspond, in many instances, in shape, size, and appearance, to the lymphocytes of the blood. There are foci and areas of cell infiltration composed almost entirely of these cells.

In addition there are not a few cells which contain two nuclei, and indistinct or poorly preserved karyokinetic figures are found once in a while. Such cells cannot be recognized as belonging to either the one or the other of the two kinds of cells described above. There are also cells that might be regarded as transition forms between these two classes. Polymorphonuclear leucocytes are not present except as occasional single cells.

The tissue here described in general contains many small blood-vessels, the greater number of which are evidently of new formation. Embryonal capillary loops can be found in very many places, and the location and the number of the vascular channels with thicker walls indicate that they also are newly formed structures. The endothelial cells are often very large and oval and are provided with swollen nuclei. It may be stated at this point that while many of these vessels occur in close proximity to the giant cells to be described, yet the most thorough examination of the series of sections has failed to disclose a single instance in which one could say, with reasonable certainty of being correct, that the giant cells played a direct part in the formation of the vessels. Direct connection between the lumen of the vessel and the giant cell or its remnants could not be demonstrated.

In some places the vessel wall is diffusely thickened and very small vessels may appear almost completely obliterated.

Tubercle bacilli were conscientiously searched for in a large number of suitably prepared specimens. In the sections stained with hæmatoxylin and carbolfuchsin some of the well preserved giant cells contain minute sausage-shaped and irregular forms that are colored black and resemble somewhat the bodies described by Metchnikoff, Welcker and others as changed and degenerate bacilli; but it would be difficult in these specimens to differentiate between such masses and nuclear fragments. In some of the sections stained with carbolfuchsin and methylene blue be-

tween the cells are found small aggregations of bright red granular masses containing bodies not unlike cocci, and also larger irregular forms that may be altered tubercle bacilli or remnants thereof, as well as a very few quite typical bacilli; isolated bacilli were also found between the cells, but only in very occasional instances.

The giant cells (Figs. 1–4, Plate III).—Imbedded indiscriminately in the granulation tissue are, as stated, a remarkably large number of variously appearing multinucleated giant cells, which, speaking generally, seem well preserved and free from evidences of extensive degeneration. The giant cells may in some instances seem small and appear immediately surrounded by ordinary young connective tissue; or they may lie in small cavities, as it were, a distinct though irregular space separating the cell from the surrounding tissue; more frequently, perhaps, they are immediately encircled by very loosely arranged cells with a tendency to a concentric arrangement, the whole resembling to a certain extent, on first view, a miliary tubercle; there are also small foci occupied by loosely scattered cells with an imperfect, concentric peripheral disposition, producing sometimes an indistinct central lumen or space, which may be quite empty, or in which, as well as in its walls, may lie giant-cell remnants, nuclear fragments, and endothelioid nuclei imbedded in protoplasmic masses similar to those of the fragmented giant cells with which they may or may not be connected.

The protoplasm of the giant cells is often almost homogeneous, or it may be uniformly finely or, in parts, more coarsely granular; the more coarsely granular parts are, as a rule, found near the centre of the cells and may contain, when more extensive, irregular chromatic fragments, while the surrounding nuclei may stain quite well. Generally the body of the giant cells sends out irregular and branching prolongations that pass among the surrounding cells and seem to fuse inseparably with the homogeneous or granular intercellular substance; at times, however, the outlines of the cell are very sharp, distinct and regular. Under all circumstances, but more particularly in the latter instances, the main protoplasm of the giant cells stains a little more deeply with eosin than the intercellular substance, and on this account it is possible to trace the origin of the many larger and smaller multinucleated and uninucleated fragments into which the giant cells are often separated. In many instances the giant cells are vacuolated; those vacuoles are produced in part by nuclei that stain very faintly; especially is this the case when the borders of the nucleus only take the stain, the vacuoles being then quite distinct in spite of their small size. In part the vacuoles seem to result from the disappearance of the protoplasm of the giant cell im-

mediately about round nuclei, which are often deeply stained; the vacuoles sometimes contain nuclear detritus and occasionally they are very large (Fig. 4 B, Plate III).

The size and the outline of the giant cells naturally vary much. In the various series of sections the majority of the cells can be traced through a very considerable number of specimens, and in this way it can be estimated that in some diameters many cells may equal as much as 60, 80, 100 and even 200μ or more; it may also happen that the other diameters are not very much less, and consequently in some sections the giant cells are almost recognizable with the naked eye. On account of the branching prolongations usually present, the outline, as a rule, is very irregular. At the periphery of many cells, and especially at the poles, there are often found scattered cells and cell masses that have nuclei and protoplasm similar to those of the main giant cell, and that consequently are either in the act of fusion with or separation from the giant cell.

The number and the arrangement of the nuclei also vary greatly; cells are observed with probably hundreds of nuclei. In a large majority of the comparatively unchanged cells studied, the nuclei are arranged in the manner characteristic of the typical Langhans giant cells, and form a more or less complete circle near the periphery of the main part of the body of the cell. They often seem to have the long axis vertical to the surface of the cell. The nuclei are usually quite small, oval or spindle-shaped; they are often rather poor in chromatin substance and stain somewhat faintly; not infrequently they are vesicular, and when only the borders of the nucleus stain, and that rather faintly, the nucleus may appear like a distinct vacuole; at other times the giant cells contain oval or round nuclei that are rich in chromatin and stain deeply. The nuclei also frequently present very atypical forms; they may be dumb-bell-shaped, flask-shaped, or very long and drawn out; budding processes connected with the main nucleus by a slender thread or stem are also present, and irregular, bizarre shapes are not infreqeunt. Occasionally fairly well preserved karyokinetic figures are also found in a single nucleus of a giant cell. In some cells there are also found, as already stated, nuclear fragments and detritus.

While the above description would answer in a general way, yet closer examination with the high powers of the microscope reveals also many other interesting appearances in the nuclei and the protoplasm of the giant cells that, although presenting difficulties in the way of concise and systematic description, as well as satisfactory interpretation, nevertheless merit some attempt thereat.

Division of the giant cells with nuclear disintegration.—It has been

mentioned that a considerable number of the giant cells seem to be in the course of division into or, possibly though not probably, fusion with smaller multinucleated masses and uninuclear cells. At times this process is associated with the formation of more or less detritus of nuclear origin and a splitting up of the cytoplasm into more minute fragments, together with the appearance of cells or phagocytes that are loaded with the remnants. Such manifestations are most noticeable in the vicinity of foci of caseation, but they are also found elsewhere. It is also observable that the bodies of the cells and cell fragments under these conditions are usually more coarsely granular. In many cases the giant cells seem to have split up into two or more separate and unequal parts, and the centre of the place that was occupied originally by the giant cell may be strewn over with nuclear fragments, occasional polymorphonuclear leucocytes and other cells filled with detritus. In other places the interior of the still intact or partially fragmented giant cell may contain much nuclear detritus, as well as vesicular and more deeply stained nuclei, while there may be arranged at its periphery cells with deeply staining nuclei which lie in more or less distinct lacunæ in the surface of the cell that then has a scalloped appearance; in a few cases these cells seemed to contain in their interior smaller and larger pieces like the substance of the body of the giant cell. In this way there may be formed, as it is easy to trace in the serial sections, a small area in which the matrix stains brighter red than that of the tissue immediately around it; the area is occupied by rather loosely scattered cells with the appearance of a concentric arrangement, especially at the periphery, producing here and there an indistinct, irregular central space or small cavity without any direct connection, however—and this was looked for very closely—with the blood-vessels. The small cells are usually oval, curved, spindle-shaped, or irregular, with a reddish, finely granular, frequently indistinct protoplasm and spindle-shaped or oval, quite lightly stained nuclei; distributed among these cells may be other round or oval cells with more chromatic nuclei and also a varying amount of free and intracellular small, deeply colored nuclear fragments, often shaped like cocci or rods; furthermore, there are not infrequently found irregular portions or remnants of the giant cells with typical or with very peculiar, flask-shaped, club- and dumb-bell-shaped nuclei, and also cytoplasmic fragments without any nuclei; polymorphonuclear leucocytes are present in rather insignificant numbers, but more particularly when the disintegration is rather well marked. Often such a mass as above outlined may be surrounded by a small capsule of a more condensed tissue, and under the low power many of them may resemble miliary tubercles.

In the above described process the amount of nuclear disintegration, the irregular splitting up of the giant cells, the coarseness of granulation of their protoplasm, and the presence of phagocytes all indicate that one is dealing with structures that are undergoing retrogressive changes and disintegration. But even in these cells there are abundant evidences of the fact that not all the nuclei of a partly necrotic giant cell are doomed to destruction. In many places the appearances point to a direct separation of apparently viable cells from the giant cells; the nucleus in the part wholly or partly separated may become rich in chromatin and stain deeply; one can also observe that the nuclei in the interior of a disintegrating giant cell, or of a fragment thereof, become more deeply stained, surrounded with a faint rim of protoplasm outside of which a vacuole with a faint network may form (Fig. 4 A, Plate III); now, when a number of nuclei undergoing such a change as this are arranged across a giant cell there may result a complete separation of the cell into unequal parts; during the time that some nuclei in the giant cell are undergoing progressive changes, others have suffered disintegration, as shown by the irregular chromatin fragments scattered about. At the poles and circumference of such giant cells there are also frequently found groups of more or less deeply stained nuclei, surrounded by a small amount of protoplasm, that give the impression of having become separated from the giant cell.

Division of giant cells without much disintegration (Figs. 2 and 3, Plate III).—There remain to be described a large number of giant cells which seem to have separated, or to be separating, into a number of small cells with but slight or no evidences of concomitant nuclear necrosis and disintegration of the protoplasm.

In quite a number of giant cells, with a finely granular cytoplasm and a more or less perfect peripheral wall of rather faintly stained nuclei, there is found a varying number of deeply stained round nuclei, many of which may lie in vacuoles in the cell substance, others upon the protoplasmic processes of the giant cell, while still others may be recognized immediately outside the cell and provided with a cell body (Fig. 1, Plate III). An apparent connection between the lightly and deeply stained nuclei can be traced through transition stages. Rather poorly preserved karyokinetic figures are also present in a very few of these cells; budding processes are also seen arising from some of the nuclei. Towards the periphery and the poles of such a cell there are found quite a number of deeply stained nuclei with rather indefinite cell bodies and still enclosed within the body of the old giant cell. The nuclei of some of the new cells, especially the ones that are free, may assume a spindle-

shaped form. There are no appearances indicating that cells are in the act of wandering into the giant cell from without. Polymorphonuclear leucocytes are not, as a rule, seen in the vicinity of giant cells undergoing such changes as these, and there are but few nuclear fragments. When these changes have advanced throughout the whole giant cell, or throughout a whole segment of one, there is formed a focus of loosely scattered oval and spindle-shaped cells with deep or lightly stained nuclei, occasionally presenting mitotic figures, and with indistinct cell bodies situated in a loosely meshed stroma; immediately around such a centre may be a more closely arranged wall composed of cells imbedded in a distinctly fibrous matrix. In general, these areas, in some part of which portions of the giant cell are usually still present, remain distinguishable from the surrounding tissue also by the fact that they have a more reddish intercellular substance, that they are not so cellular and that the nuclei of the cells are less deeply stained. In the most advanced districts, where the fibrillation of the young connective tissue is most marked and the cellular infiltration less pronounced, the areas described are usually small and wholly occupied by cells, many of which cannot be distinguished, as regards shape, from the cells in the recent fibrous connective tissue around the area. Appearances indicating the transformation into blood-vessels of such districts of cells derived from the disunited giant cells are not present.

Undoubtedly the loosely filled spaces above referred to would later on become filled or obliterated in part, perhaps, by cell proliferation, but principally, most likely, by contraction of the intercellular substance.

SUMMARY.

From the foregoing description of the histological changes in the leptomeninx it is quite evident that we are dealing with a chronic, stationary, healing form of tuberculous inflammation.

This statement is substantiated, in the first place, by the clinical history. The only reasonable interpretation of the symptoms would establish the duration of the process as four months. The imaginable contingency that there existed first a meningeal syphilitic lesion that was dispersed by the iodide of potassium only to be followed by a tuberculous infection is so remote and unlikely that it need not be discussed. At all events the tuberculous leptomeningitis, which presented a typical distribution, began insidiously, existed at times in a latent condition, and pursued a very anomalous course, marked by a

relative mildness of all the symptoms, and thus it came about that when an apparent or real improvement followed the administration of iodide of potassium able observers were induced to make an erroneous diagnosis. Death occurred as a result of an intercurrent infection.

The long duration of the process is also shown, anatomically, by the thick layer of firm, translucent and gelatinous material that matted together the structures at the base, and also by the evident adhesions between the pia and the brain.

The histological examination furnishes proof positive of the correctness of the conclusion in regard to the peculiar character of this process because it shows:

(1) That the tuberculous proliferation is uniform in development and has reached nearly the same stage of evolution throughout the entire extent of the leptomeninx involved; it is not a process that has advanced by exacerbations and irregular extensions; the lesions are, generally speaking, of nearly the same age everywhere and must have begun at about the same time.

(2) That only a very limited degree of caseous degeneration is present, pointing to an early arrest of the activity of the tubercle bacillus or to a very decided diminution or attenuation of its virulence.

(3) That the subendothelial intimal proliferations of epithelioid cells, so generally found in acute tuberculous leptomeningitis,* have in this case become more or less completely changed into distinct fibrous tissue in which but very slight, if any, direct evidence of its tuberculous origin can be found. It is only by recognizing that the chronic endarteritis is most marked in correspondence with the most advanced adventitial tuberculous changes, and by finding an imperfect, much altered giant cell in one district of intimal thickening, that we were able to establish the direct kinship of the endovascular changes with those of the pia in general.

(4) That acute inflammatory changes, in the form of emigration of polymorphonuclear leucocytes and of fibrinous exudation, are en-

* See an article by the writer entitled "The Vascular Changes of Tuberculous Leptomeningitis, especially the Tuberculous Endarteritis," in this Journal, Vol. I, No. 1, 1896.

tirely absent in all parts of the district involved. The presence of a turbid serous fluid is of course not at all inconsistent with the view that the anatomical changes are of long duration.

(5) That the granulation tissue present is, in general, undergoing fibrillation and contains a rich supply of embryonal capillary vessels as well as of larger blood-vessels of evidently new formation. The absence of any considerable extent of polymorphonuclear leucocytic infiltration in this tissue has already been referred to. The cells in the granulation tissue correspond to the cells of embryonal or formative connective tissue. Vacuolation is rarely present.

(6) That the unusually large number of giant cells present are remarkably free from evidences of necrosis and degeneration of the character ordinarily observed in tuberculous proliferations, that they do not contain in demonstrable form tubercle bacilli, and that the majority of the giant cells seem to be separating into individual cells and smaller masses often with, but sometimes also without, evidences of nuclear disintegration. The possibility that these phenomena may signify fusion instead of the sundering of cells will be discussed below.

For these reasons there can be no doubt that the general claim that we are dealing with an instance of chronic, healing tuberculous meningitis must be regarded as established beyond dispute.

The growth of tubercle bacilli in the glycerine-agar tubes, inoculated with the fluid from the pial meshes, and the demonstration of tubercle bacilli, though in very small numbers, between the cells of the embryonal tissue, furnish the positive evidence that we are actually dealing with a tuberculous process due to living and not to dead bacilli.

The degree of virulence of the cultures of tubercle bacilli was, unfortunately perhaps, not studied. The presence of living tubercle bacilli in a tissue free from active and acute changes characteristic of tuberculosis demonstrates that, whatever the actual degree of virulence of the bacilli may have been, the tissue in which they were found was at this time relatively immune from their action. The manner in which this immunity was produced, and in which the process of healing was initiated, need not be discussed at this time any further than to again direct attention to the fact that the bacilli lost

their virulency as regards the cells in this leptomeninx before these cells underwent any marked degree of degeneration. The cells of the tuberculous proliferations survived the further action of the bacilli whose original effect it was to initiate cell accumulation or proliferation; the cells also retained sufficient vitality to develop, in some instances at any rate, into formative cells according as their origin would dictate, *e. g.* into fibroblasts. That fibroblasts are formed only by embryonal connective tissue cells, and not by wandering cells, such as the large mononuclear leucocytes, we are well aware, is possibly still a disputable assumption, and we do not consider it pertinent to discuss the question any further in connection with this study, but would only emphasize the point that some of the cells of tuberculous proliferations may, under favorable circumstances, become formative cells, and, furthermore, that the amount of formative tissue produced may be far in excess of what is actually needed for purposes of repair only. Surely the appearances here noted indicate that the bacillus of tuberculosis has the power to stimulate fixed cells to multiply, unless one assumes that all, or almost all, the formative cells here seen are derived from wandering cells attracted by the presence of the bacillus and its products.

As to the ultimate fate of the formative and other cells in this healing tuberculous tissue no final statements can be made. It must be remembered that it is only one stage in the process of healing that is dealt with. The well marked evidences of fibrillation, the quite extensive formation of new vessels, the absence of evidences of degenerative changes in the uninuclear cells, all point to the production of new fibrous tissue as sure to occur, but it seems quite probable that occasional epithelioid cells may undergo or have undergone dropsical or other forms of degeneration, although it is certainly apparent that so far as the small cells are concerned the involution of the tuberculous tissue is not occurring through disintegration.

Perhaps the most interesting feature in this case is the opportunity it affords to study the changes in the giant cells of healing, non-degenerated tuberculous tissue. In the first place, the large number of giant cells is quite remarkable. The general characters of the tissue

in which they are found recall the fact that giant cells are regarded as quite constant elements in chronic mild tuberculosis; often the giant cells are the only cells that contain bacilli (Koch). In this instance the giant cells do not contain bacilli that are demonstrable by the usual methods; neither do they contain bodies that can be definitely interpreted as degenerate forms of bacilli such as those found by Metchnikoff, Stchastny, Welcker, and others, in the giant cells of Spermophilus guttatus, in avian and in human tuberculosis. Metchnikoff states, however, that he knows of the occurrence of such degenerate forms only in the Spermophilus guttatus under the circumstances mentioned, and in the rabbit and guinea-pig in mammalian tuberculosis, but not in man; consequently, the manner in which the giant cells rid themselves of the bacilli undoubtedly present in their interior at some time during their existence, must as yet remain without any explanation.

In the description of the histological changes the various appearances presented by the giant cells are described somewhat minutely. The essential observations made concern, in my opinion, the further fate of giant cells which are still found to persist in healing non-degenerated tuberculous tissue. It was, I believe, quite conclusively shown that the consecutive changes appear to consist in the breaking up of the nuclei, the removal of the detritus by phagocytes, and the formation of a few apparently viable uninuclear cells in the case of more degenerated, exhausted giant cells, while other, and, as it would seem, better preserved or younger giant cells, separate into a number of individual, uninuclear cells with but little or no nuclear disintegration.

Objection might be raised to this interpretation of the appearances in the giant cells. While no one could very well dispute the view that part of the giant cells are undergoing retrogressive and absorptive changes with the production of some viable cells, a question might well be raised concerning the nature of the process taking place in those giant cells that have been spoken of as splitting up or dividing into uninuclear cells and smaller multinucleated masses without much evidence of nuclear disintegration. It might be claimed that the pro-

cess is one of fusion of many cells to form giant cells, and not one of division of fully formed giant cells into small cells. But a broad view of the processes described speaks against fusion. In the first place we are not dealing with a stage of tuberculous proliferation (Baumgarten), or cell accumulation (Metchnikoff), in which one would look for the production of giant cells, no matter which view concerning the histogenesis of tubercle be assumed as the correct one, because it has been demonstrated that, from whichever point of view the lesions are examined, the same positive conclusion that they are in the process of healing is reached; there is, therefore, no occasion for the formation of new giant cells in such wide-spread degree throughout the district involved.

It might be claimed that the cells became arrested and, as it were, fixed in the act of fusion which was taking place in the early stage of the meningitis, but it would be difficult to understand the nature of the stimulus that could hold the cells together in such a peculiar manner for such a long time. It must be remembered that bacilli or bacillary detritus could not be found among the incomplete or in the complete giant cells.

In the second place the difference between the cells that are undergoing disintegration and those regarded as dividing is essentially, to a certain extent at any rate, one of degree, because in the first instance there is not much, if any, doubt but that viable smaller cells are also formed, and in the second instance some, though often very slight, evidence of nuclear fragmentation is nearly always present; it would also be correct to infer that in advanced subdivision of a giant cell much, and perhaps all, of the nuclear detritus produced might have been removed up to the last trace; finally, the two extremes of these changes in the giant cells are connected by transition stages passing by gradation from the one to the other. Hence it is justifiable to conclude, for the time being, that *in healing non-degenerated tuberculous tissue, the multinucleated giant cells may in part disintegrate and undergo absorption, in part form viable small cells; that both these changes may, and usually do, affect the same cell, but that in one class of cells—presumably the older or the more exhausted—the retrogres-*

sive process is predominant, while in a second class of cells—presumably the young and vigorous—the progressive changes are the more marked.

In this connection it may be pointed out that while there cannot very well be any question but that we are dealing only with dividing and not coalescing cells, yet if this conclusion should be disputed and found incorrect, then the only remaining alternative would be to infer that this tissue furnished a unique and striking example of the formation of plasmodial masses by fusion in human tuberculosis, a conclusion to which many pathologists would refuse to subscribe, if for no other reason than because it is not in accordance with the almost universally accepted teachings of Baumgarten and Weigert in regard to the mode of formation of the giant cells in tuberculosis.

Believing as I do that the giant cells under consideration are in the act of division and not at all of fusion, there remain to be discussed some of the histological and other features presented by the dividing cells.

Many of the giant cells, perhaps the majority, contain larger and smaller vacuoles in the protoplasm. The exact significance of this vacuolation is not always clear. When the vacuolation accompanies an evident solution of the nucleus (karyolysis), there cannot be any doubt but that we are in the presence of a distinctly retrogressive process. Vacuoles are also most numerous in the giant cells that present other evidences of degeneration, such as coarseness of the granules in the protoplasm and extensive nuclear disintegration, but they occur as well around nuclei that stain deeply, around cells that seem to be separating from the giant cell, and even about nuclei that present mitoses. The formation of vacuoles seems to be responsible, to a certain extent at any rate, for the diminution in the volume of disintegrating and dividing giant cells, as shown by the clear spaces that form about them; these spaces are too large and occur too uniformly to be attributed solely to artificial shrinking produced by the hardening in alcohol.

Further undoubted evidence of retrogression in certain giant cells is the occurrence of nuclear disintegration, or karyorhexis, which sets

free larger and smaller chromatin masses that are recognized in the giant cell as well as in the interior of the phagocytes usually found around such cells.

Almost all the polymorphonuclear leucocytes found in this tissue are met with around giant cells with broken-up nuclei. In many nuclei of disintegrating giant cells can be noted appearances that correspond well to certain stages in the complicated karyorhexis observed in anæmic necrosis by Schmaus and Albrecht; some of the nuclei with budding processes correspond particularly well with those in certain of their drawings; the interior of giant cells of tuberculous tissue may, it would seem, present conditions favorable to the development of this series of postnecrotic nuclear change. Vacuolation, karyolysis and karyorhexis are the essential steps that lead to destruction of the whole or parts of some of the giant cells; associated with these processes there is usually observed a splitting up of the body of the giant cell into irregular fragments with as well as without nuclei; and, as described, more or less phagocytosis of the resulting remnants of various kinds is seen.

But evident degenerative and necrotic processes in a giant cell may be associated with progressive changes. While some nuclei undergo vacuolation or break up, others seem to become richer in chromatin and to stain more deeply at the same time that they seem to acquire cell bodies quite distinct from the protoplasm of the giant cells; this hyperchromatosis does not, therefore, seem to be a stage in karyorhexis. A very few but undoubted karyokinetic figures were found, together with evidences of division of the cell body formed in the giant cell protoplasm. Precisely similar changes are described by Klebs in healing pulmonary tuberculosis of the guinea-pig; the nuclei of the giant cells became rich in chromatin and karyokinetic figures occurred. Krückmann among others has found occasional mitoses in giant cells around foreign bodies, as well as elsewhere, but it would seem that such mitoses have always been interpreted as indicating the probable mode of formation of the giant cells rather than of their involution.

The question of mitosis in existing multinucleated cells has recently been studied by Krompecher, who concludes that the individual nuclei

of such cells may undoubtedly divide by mitosis, either simultaneously or at separate times. Division by amitosis can also occur, but mitosis is the only progressive form of division, amitosis being a retrogressive, disintegrating process that must be looked upon as an evidence of degeneration of the nucleus. Ziegler states that in division of giant cells whose nuclei have multiplied by mitosis it may happen that the separating cell remains enclosed in the protoplasm of the mother cell.

A singular phase in the involution of the giant cells in this pia is to be found in the existence of progressive changes side by side with nuclear necrosis and with degeneration; this finding indicates that giant cells may contain many independent elements which, though apparently fused into one large cell, may preserve their individuality so that while some nuclei die, others proliferate and perhaps feed on the remnants of their dead brethren and form new, viable small cells. The nuclei in giant cells may be looked upon as representing independent centres, capable at times of existing even though the cell protoplasm is disintegrated. Many of the giant cells separate into individual cells, unaccompanied or unassociated with much evidence of necrosis. These cells may be regarded as the more vigorous forms. Here also are observed occasional mitoses—but on the whole extremely few—and very constantly an evident increase in the amount of chromatin in the nuclei of the new cells as compared with the amount ordinarily found in the nuclei of giant cells.

These deductions concerning the persistence of the vitality of some of the nuclei, even in the presence of molecular and morphological changes in the cytoplasm and in other nuclei of the giant cell that lead to disintegration, are not entirely without the support of previous observations on cells, which, although made under different conditions, are nevertheless, it would seem, applicable to cells in general. Thus the brilliant investigations of Loeb upon the effects of various unfavorable surroundings, such as absence of oxygen or reduction of the amount of water, upon the cleavage of eggs of many kinds, show that the conditions which arrest development are qualitatively alike for nucleus and protoplasm, but quantitatively less for the protoplasm; when the irritability of the protoplasm is suspended the nucleus may

segment without segmentation of the protoplasm, but upon re-establishment of favorable conditions the protoplasm may divide into about as many spheres as there are nuclei preformed—the nucleus persists, preserves the irritability of the cell and stimulates the protoplasm to segmentation. From the appearances of the giant cells here described it would seem, then, that some nuclei are able to maintain their vitality longer than others in the same cell, and under certain conditions to stimulate parts of the protoplasm to segment; in other cells all the nuclei have, as a rule, preserved their irritability.

The groups of cells formed by the dividing of the giant cells can be traced by studying the process at the different stages in the different parts of the tissue. They assume an oval or spindle-shaped form, becoming more and more like the formative and endothelioid cells of young connective tissue, but their ultimate fate cannot be determined because it concerns essentially only one limited period in the involution of the tissue. It may be said with reasonable certainty, however, that the new cells do not form blood-vessels, but as regards their forming lymph-vessels nothing definite can be concluded.

It would not be safe to draw any definite conclusions, from the appearances described, with regard to the origin and the mode of formation of the giant cells. The resulting small cells in general resemble very much endothelial and formative cells, but some of them are, at certain stages at any rate, not unlike large mononuclear leucocytes; their final fully developed or mature condition being unknown, no positive inference can be drawn as to their pre-giant-cell origin. The evidence points to the fact that the most probable origin of the giant cells, as indicated by their form and the apparent future career of their descendants, would be the fixed mesoblastic cells of the pia. In regard to the mode of formation of the giant cells it is quite clear that it must involve some process which is not incompatible with the viability of the small cells which may spring from the giant cells. Whether this would speak more in favor of formation by fusion than by karyokinesis of a single cell without division of the cell body cannot be well determined, and as long as authors are not agreed upon the question of the production of living, procreative cells by amitosis

(direct segmentation, direct and indirect fragmentation) it would not be profitable to discuss the compatibility or incompatibility of the views of those investigators who trace the origin of giant cells to amitotic division, with the progressive changes that giant cells have been shown to be capable of.

The fact that giant cells in tuberculous tissue, under certain conditions, undergo progressive changes and separate into small, living cells proves that they are not, as claimed by Baumgarten, Weigert and others, necrobiotic elements that are doomed to destruction from their very inception. On the other hand it lends more strength, if that were necessary, to the teleological view urged by Metchnikoff that they are living, defensive cells (whatever their origin may be), formed for the distinct purpose, like plasmodial masses in general, of isolating and removing foreign, harmful bodies, in this case the tubercle bacillus, and, having accomplished their object without being destroyed or exhausted, or the cause of their formation being removed or neutralized in some way, they, or their nuclei, may retain enough irritability to form a larger or smaller number of living, small, uninuclear cells.

I wish to thank my friend Dr. J. D. Freeman for his excellent drawings.

DESCRIPTION OF PLATE III.

Fig. 1.—A giant cell in one portion of which many of the peripheral nuclei are rich in chromatin and stain deeply; there are vacuoles about some of these nuclei. To the left is a small fragment that has separated from the giant cell proper and which contains a few deeply staining nuclei as well as an indistinct cell with two nuclei. Zeiss, obj. 4°, ap. 0.95 apochr.; oc. 8 (compensat.); tube-length 160.

Fig. 2.—The same giant cell as in Fig. 1, but at a different level. The cell is separating into uni- and multinuclear cells and masses of varying forms. Magnification same as Fig. 1.

Fig. 3.—In the central part of the drawing are spindle-shaped and irregular cells and masses derived from the splitting up of a giant cell, as shown by study of the serial sections. Same as Fig. 1.

Fig. 4.—*A*, a section of an irregular, finely granular giant cell with faintly stained and vesicular nuclei, some chromatin granules, occasional deeply staining nuclei; and lying in a vacuole a distinct cell, the nucleus of which seems to have divided and the cell body of which presents a central constriction as though about to divide. At the lower margin of the cell are

separated protoplasmic masses, one of which contains a deeply stained nucleus.

B, giant cell with huge vacuoles, containing fine granules. Same as Fig. 1.

Fig. 5.—Chronic fibrous thickening of the intima.

A, subendothelial fibrous tissue covered by closely arranged nuclei.

B, imperfect or dividing giant cell (?) in the deeper, more cellular part of the thickening.

C, intact elastic layer.

Adventitia the seat of considerable infiltration. Zeiss, apochr. obj. 4°; ap. 0.95; oc. 4 (compensat.).

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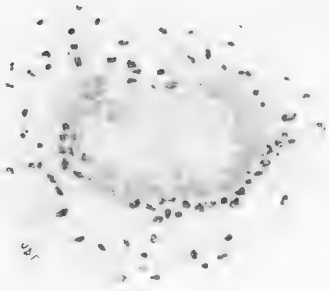

FIG. 1.

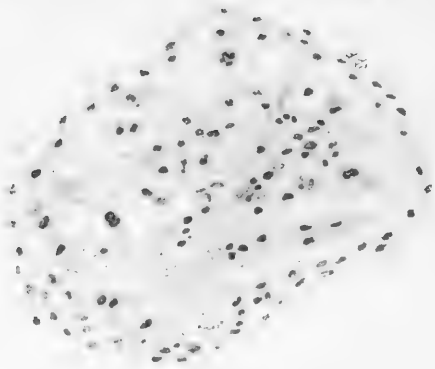

FIG. 2

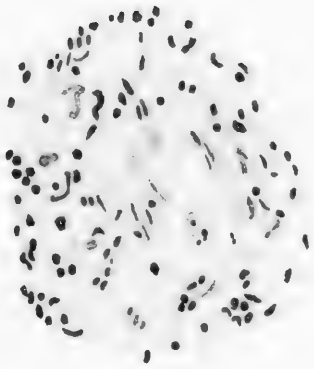

FIG. 3.

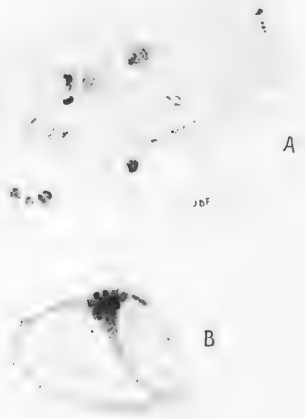

FIG. 4.

FIG. 5.

A CASE OF PSEUDO-LUPUS VULGARIS CAUSED BY A BLASTOMYCES.

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Plates IV–VIII.

Within the last three years a number of important communications have appeared concerning pathogenic yeasts. Before this period it was generally believed that among the yeast plants there existed none pathogenic for man or animals. The experiments of Bernard, Grohe, Popoff, Falk, and the more recent ones of Neumayer and of Raum, furnished no satisfactory evidence in favor of the possession of infectious properties by the blastomycetes.

In August, 1894, Busse * published a brief report, followed in April, 1895, by a fuller account of a case interpreted by him as one of chronic pyæmia, in which he found in tissue removed during life and in diseased foci after death, numerous parasitic bodies which he proved to be yeast fungi. These he isolated in pure culture and inoculated successfully into animals. He designated the disease produced by this parasite in man as saccharomycosis hominis.

In May, 1894, three months before Busse's first publication, one of us (Gilchrist) exhibited and described before the American Dermatological Association in Washington, microscopical sections from a case of cutaneous disease in Philadelphia under the care of Dr. Duhring, who had excised and sent a piece for microscopical examination. Peculiar parasitic bodies, which were interpreted as plant rather than animal forms, were observed and described. After the appearance of Busse's paper these bodies were recognized as forms of blastomycetes. This case has been fully described and illustrated by Gilchrist under the title of "A Case of Blastomycetic Dermatitis in Man."

* References to literature will be found at the end of this article.

The most prolific writer upon the subject of pathogenic yeasts since the year 1894 has been Sanfelice, who has obtained from various sources cultures of blastomycetes pathogenic for animals. A systematic experimental investigation of the pathogenic properties upon animals of yeast cultures obtained from various sources has been made also by Rabinowitsch. The Italian investigators especially, of whom, besides Sanfelice, may be mentioned Maffucci and Sirleo, Roncali, Aievoli, Corselli and Frisco, Fermi and Aruch, d'Anna, Binaghi, Secchi, Rossi Doria, Casagrandi and de Simoni, have interested themselves in this subject. Many of these writers, as well as Kahane in Vienna, deal with the question of the parasitic origin of malignant tumors. Although it cannot be doubted that in some instances yeasts have been cultivated from such tumors by Roncali, Corselli and Frisco, and others, we do not consider as thus far successful the efforts to identify the much discussed cell-enclosures in carcinoma and sarcoma with blastomycetes or other genuine parasites.

Of interest are the observations of Tokishige concerning the blastomycetic origin of a farcy-like disease of horses and cattle in Japan, apparently identical with a similar disease in Italy and France likewise referred to blastomycetes by Fermi and Aruch, and also those of Sanfelice concerning so-called epithelioma contagiosum (Bollinger) of fowls, which had been attributed by Rivolta and Delprato to psorosperms, but which Sanfelice believes to be caused by a yeast parasite.

Curtis, in France, has published interesting observations upon a yeast parasite, which he calls Saccharomyces subcutaneus tumefaciens, found in a tumor of myxomatous appearance in the thigh of a human being. This he cultivated and inoculated successfully into rats, mice and dogs.

A preliminary account of the case which forms the subject of the present paper was published in the Bulletin of the Johns Hopkins Hospital in July, 1896. Since that time we have had the opportunity of making more extended observations and inoculation experiments, and are, therefore, now in a position to give a full description of the case and the results of our investigations. The patient first came under the care of Professor Halsted of the Johns Hopkins Hospital, by

whose permission one of us (Gilchrist) had the opportunity of examining and studying the case. We take this opportunity of thanking Professor Halsted and also of expressing our indebtedness to Professors Welch and Flexner for their valuable aid and advice in our investigations.

Clinical History.—The patient is 33 years of age, married, about 5 feet 9 inches in height, and of a slender but wiry build. He is one of thirteen children, twelve of whom are still living and in good health; one child died when five months old from whooping-cough.

On July 4, 1885, the present eruption is said to have been first noticed at the back of the left ear, just behind the mastoid process, as a pimple which was about as large as a grain of wheat. It was roundish in shape and only slightly raised; the pimple soon became pustular. The eruption began to spread in a linear direction towards the lobe of the ear, but increased in extent very slowly, so that during the succeeding four or five years the length of the patch was only from 3 to 5 cm. As the disease extended the oldest portion gradually healed spontaneously, and a white, slightly hypertrophic scar was produced. The healing process was always only partial and took years for its accomplishment. The linear scar, which is now distinctly visible, is about 4 cm. long and 5 to 6 mm. broad.

After four or five years the disease, which continued to spread very slowly forward, began to involve the cheek, and then gradually extended upwards and downwards, so that seven years after the first appearance of the eruption it had only reached the external canthus of the left eye, after which it continued to travel along the eyelids. The tendency to heal as it progressed was still a characteristic feature of the process, but the scar was now more atrophic, whitish and thin, particularly over the cheek. Besides extending to the eye, the lesion also spread gradually down the left cheek until it reached the left side of the chin. In about nine years the nose was attacked by the growing edge; the bridge was soon crossed and the right eyelids and eyebrow became involved. After this the patient noticed that the growth became more rapid, especially on the forehead and down the right cheek.

About one month after the first lesion was observed there appeared on the back of the right hand, over the middle of the third metacarpal bone, another pimple which soon became pustular and opened spontaneously. This lesion also began to increase slowly; it remained superficial and assumed the same characters as that on the face; it took about four years for it to spread over the whole posterior surface of the hand, from the

knuckles to the wrist, and from the thumb to the inner margin of the hand. The eruption was treated successfully with caustics by the family physician.

Another secondary lesion occurred six months after the first on the right side of the scrotum at the upper portion, and this again, as was stated, had the appearance of a pimple which followed the same course as the others, except that it contained only a little pus; but the characteristic creeping propensities were shown until the area attained to about the size of a silver dollar. This scrotal lesion grew slowly for a year, when it healed spontaneously. No external applications were used.

Still a fourth lesion appeared in the same manner and at about the same time as the scrotal eruption, on the anterior surface of the left thigh and just above the internal condyle. This also spread peripherally for about a year until it formed a patch 5 cm. by 7.5 cm. in size, when it also healed spontaneously without treatment, leaving a slightly raised scar. On the back of the neck, just above the 7th cervical spine, a fifth lesion made its appearance a little later than the one on the thigh and continued to grow for two years, when it also healed spontaneously. The patient says none of the lesions were at all painful. Upon entrance of the patient into the Hospital it was noted that on the face the diseased portion presents a distinct line of demarcation. The upper border, as shown in the photograph (Plate IV), extends from the middle of the right eyebrow sloping upwards and then passes across the forehead to the left side, where it curves down again to a point just outside the external canthus of the left eye. This border, with the exception of a small portion which has apparently healed, consists of a comparatively thin (8 mm. wide), inflammatory, red ulceration, which is superficial and covered with a scanty scab. Along the right eyebrow the characters of the lesion present a somewhat different aspect, showing a firm papillomatous appearance. These papillomatous growths can be separated, and sometimes a minute quantity of pus can be squeezed out from between them. Thin scabs are found partially covering these lesions.

The right border of the facial patch extends from the right eyebrow horizontally outwards to a point within an inch of the ear; it then proceeds downwards and forwards towards the right angle of the mouth, and stops abruptly within 1.5 cm. of this point. The border of the patch then turns slightly upwards and inwards towards the ala of the nose. This right border is about 2.5 cm. broad and presents, especially towards the lower angle, a pronounced papillomatous character, being dry and scabby; where it has encroached on the region of the beard it is pierced by hairs. The largest papillæ, which are all closely aggregated together,

are about the size of large pin-heads; from between these also sometimes a small quantity of pus can be squeezed out. The other portion of this border is covered with a thin scab, on removal of which a superficial ulcerative patch is exposed. The continuation of the lower border extends across the nose to within an inch of the tip as a superficial ulcerative edge.

From the left ala the lesion passes downwards to the left angle of the mouth, whence it extends still further down to the border of the jaw, along which it continues upward to the point of its first commencement. This area also is about 2.5 cm. broad, and again at the lower angle, *i. e.* between the mouth and the border of the jaw, the papillomatous character is well marked and numerous hairs protrude. These enlarged papillæ are raised nearly 6 mm. above the level of the normal skin. The lesion along the lower jaw is much narrower and the ulceration is quite superficial.

The whole area enclosed by this irregular border consists of one continuous atrophic scar which is whiter and thinner than normal skin. The eyelids of both eyes are practically destroyed, so that the patient cannot cover the eyeballs. Around the left eye the patch presents a raw, red, moist, easily bleeding surface, which extends for nearly 3 cm. downward from the lower border of the eye. The right eyelids present similar appearances except that the lower patch is not so extensive. The tears are continually running over these patches, particularly on the left side. The patient says that the disease is not even now particularly painful.

On the back of the right hand is an atrophic thin whitish scar which extends over the whole surface of the dorsum. The scar on the right side of the scrotum is about 3.5 cm. in diameter, is reddish and somewhat contracted, whereas the scar on the left thigh is smooth, thin, very white and not contracted. The lesion which was situated on the back of the neck has left a hypertrophic scar of distinctly keloidal appearance.

There are no enlarged glands. The patient has no cough; the bowels are regular, tongue fairly clean; he says that his general health has always been good. There is no history of syphilis, although the patient's wife had a miscarriage at four months, but no children. The wife's menstrual courses are very irregular. No tuberculous history in the family could be obtained.

Our first impression was that we had to deal with a case of lupus vulgaris, which would, therefore, have made its first appearance at the age of twenty-two years; but the primary lesion of all the patches

presented the same feature, appearing first as a pimple and then as a pustule, followed later by a superficial ulceration spreading, in the majority of the lesions, peripherally. As the disease extended, atrophic and in one lesion hypertrophic scarring had resulted. There was no history of the formation of lupus-nodules nor could any such lesion be found. The papillomatous form of the disease was in many respects suggestive of lupus papillomatosus. Tuberculosis cutis was then thought of and particularly tuberculosis verrucosa cutis (Riehl and Paltauf); but this disease is usually due to frequent contact with decomposing animal matter and our patient had not been exposed to such influences, although clinically various points of resemblance presented themselves between this disease and our case. Tuberculosis verrucosa cutis begins as a papule, which later becomes pustular, and after forming a scab, which falls off, takes on a papillomatous growth. It spreads very slowly peripherally, and on lateral pressure a little pus can be squeezed out from between the papillæ; but the hands, forearms and feet are the only regions of the body where this disease has been found. The history of the lesions in our case, and the fact that they healed spontaneously in three situations, would appear to be clinically almost sufficient to exclude tuberculosis of the skin in any of the usual forms. Scrofuloderma was also considered, but was excluded on the grounds that the lesions in our case practically presented none of the typical features of this disease, nor were there any enlarged glands or constitutional symptoms to support this view.

For purposes of diagnosis three portions were excised, one from the right eyebrow (inner border), one from the right border (papillomatous) of the patch, and the third from the lower end of the left border near the chin. One portion was dropped into 5 per cent. formalin solution, and the other two into 95 per cent. alcohol.

PATHOLOGICAL HISTOLOGY.

Unstained sections treated with ordinary liquor potassæ showed the presence of numbers of curious bodies which were doubly contoured and very refractive, whereas the tissue assumed a blurred appearance.

The stained specimens showed a hypertrophy of the epidermis (Plate V, Fig. 1), throughout which numerous well defined miliary abscesses

of various sizes were scattered. Fig. 1, Plate VIII, represents one of the smaller miliary abscesses in the epidermis and contains two parasites. The epidermis was elsewhere considerably infiltrated with polynuclear leucocytes, and irregular masses of detritus covered certain portions of the epidermis. Large collections of granulation cells were massed in the corium, and numerous miliary abscesses (Plate V, Fig. 1, *b*) were also observed in the upper part of this region. A considerable amount of inflammatory material was also distributed throughout the corium, and in a number of sections there was distinct evidence of the formation of tubercle-like nodules (Plate V, Fig. 1, *n*) in the deeper portion of the corium.

Situated in all the miliary abscesses, and also among the granulation cells in the corium, as well as in the pseudo-tubercles, were numbers of doubly contoured, refractive, round and ovoid bodies (Fig. 1, *p*), varying in size from 10 to 20μ in diameter. Many of these forms presented buds of various sizes, and a vacuole was often, although not always, discernible. The bodies were usually found singly or in pairs, but they were sometimes arranged in groups. They were more numerous in the miliary abscesses than elsewhere. The contents of the bodies consisted of finely and coarsely granular protoplasm which took up the ordinary stains. Unna's method of staining for mastcells with polychrome methylene blue was employed on some sections and the coarse granules were found to be stained red, whereas the remaining portion of the protoplasm was practically decolorized. This reaction seemed to show that the coarse granules were basophilic.

Mastcells were somewhat numerous, but comparatively few giant cells (Fig. 1, *G*) were scattered throughout the corium; some of the latter enclosed one or more parasites. The contents of all the miliary abscesses (Plate V, Fig. 1, *a*, and Plate VIII, Fig. 1) consisted principally of large numbers of polynuclear leucocytes and nuclear fragments besides the parasites (*p*). Detached epithelial cells were sometimes present in the epidermal miliary abscesses.

Fig. 1, Plate V, represents a section from the cutaneous lesion; in it are seen numerous miliary abscesses (*a*) scattered throughout the hypertrophied epidermis (*e*) and corium (*c*). The section also shows the communication of one of the abscesses with the surface (*s*). Practically the entire epidermis was infiltrated with polynuclear leucocytes. The whole upper half of the corium (*c*) was the seat of acute and chronic inflammatory changes. Two hair follicles (*h*) were seen in the section, but they were essentially normal. The sudoriparous glands were also unaffected, and there did not appear to be any particular relationship between the diseased condition and the appendages of the skin.

Two pseudo-tubercles (*n*) were seen in this section in the lower portion of the corium; these showed at their centres one or more parasites. The pseudo-tubercle was thus made up of one or more organisms in the centre, surrounded by a number of polynuclear leucocytes, around which were three, four or more layers of epithelioid cells, while a large number of mononuclear or lymphoid cells formed the peripheral portion. The pseudo-tubercles were more numerous in the papillomatous or chronic variety of the lesions; whereas in the acute or ulcerative form, miliary abscesses, and consequently more parasites, were present.

In the papillomatous lesions the papillæ were markedly hypertrophied and the mouths of the hair follicles, especially from the region of the beard, showed pronounced hyperkeratosis (*k*). The epidermis itself was not much altered and did not present the miliary abscess-formations. The corium consisted chiefly of large masses of granulation cells with very few of the miliary abscesses situated near the upper portion. Parasites were found in all the abscesses. One could also find now and again an organism among the granulation cells, but here they were not at all numerous; giant cells were present in only small numbers.

Generally speaking every miliary abscess contained one or more parasites (*p*), which were often in a budding stage. No parasites were found enclosed in cells except in a few giant cells which were situated in the deeper portion of the corium.

The organisms were first found and studied with the ordinary hæmatoxylin and eosin stain. Under ordinary circumstances they could always be found in the tissues, but if the sections were at all thick one could easily pass them over. They took the hæmatoxylin stain just about as readily as the cell nuclei, but were more easily decolorized. We found that the most practical method of demonstrating the presence of these organisms in the tissues was to immerse the section in a mixture of equal parts of liquor potassæ and glycerine, when they were at once easily recognized. When we used Unna's polychrome methylene blue stain the organisms were very prettily stained, the capsule and protoplasm being colored blue, whereas the coarse granules were stained red. Methylene blue (aqueous) and eosin also gave good results. With carbolfuchsin and methylene blue the capsule was sometimes stained red and the protoplasm blue.

The organisms were usually round or oval, and their resemblance to the parasites found in Gilchrist's case of blastomycetic dermatitis,

already cited, was very close, although in the latter they were more numerous. The mode of development by budding could be followed out in the sections. In some instances the capsule had given way and the endosporium protruded as a bud (Fig. 12, Plate V), but more often the bud was formed by a pushing out of both endo- and exosporium (Fig. 11, *b*). The bud then grew larger and larger (Plate V, Figs. 3-13) until it reached almost the size of the parent, when the endosporium or protoplasm became divided by a clear space (*s*, Fig. 5). This stage was followed by a folding in of the capsule (*c*, Fig. 7) until the folds met and a separate organism was formed (Fig. 10). Sometimes a second bud was produced before the first had become detached (Figs. 11, 12, 13). A vacuole (see Figs. 2, 4, 6) was more often than not seen in the parasite, but there was much variation both in its size and shape. A few organisms presented rather a curious appearance (see Fig. 8); the section was stained with hæmatoxylin and eosin, but this organism did not stain, and presented, especially its central portion, a very refractive appearance, as if it had undergone some form of degeneration; surrounding the capsule was a delicate, fibrous-like structure of irregular outline. One shown in Fig. 9 also presented a similar appearance as far as the external, fibrous-looking, irregularly shaped coat was concerned, but the central portion stained in the same manner as in the other organisms. Fig. 15 shows also an unusual form where the protoplasm has contracted into a half-moon shape. Sometimes a number of parasites are collected into a group (Fig. 16), which is usually situated in the corium and rarely in an abscess. Plate VIII, Figs. 3, 4, and 5, show varieties of organisms enclosed in giant cells. In Fig. 5, Plate VIII, the vacuole, both in the parent and in the bud, was particularly clear and well marked. Four parasites were found enclosed in a giant cell as shown in Fig. 4; three of them were in various stages of gemmation.

CULTURES.

After careful sterilization of the surface of the papillomatous lesions, cultures on plain agar were taken from two places from the pus, which after pressure oozed out from between the growths. Other

similar cultures were taken from the diseased area after removal of the scab, and from one excised portion of the tissue.

Although every possible precaution was taken in order to avoid any contamination, yet the cultures from the tissues contained a profuse growth of pus organisms, and in only one instance a single large colony of the parasites developed. These cocci were not further noticed, as they were not found in the pus and could not have caused the chronic process.

Both of the tubes inoculated with the pus showed in one week a pure growth of the organism about to be described.

Morphology.—This was first observed in specimens taken from young colonies from the surface of plain agar, and mounted in salt solution. They may be studied either with the No. 7 Leitz objective or by means of the oil-immersion lens.

The most usual form consists of a large, round, oval, or irregular, highly refractive body, surrounded by a doubly contoured membrane, consisting of an exosporium and an endosporium (Plate VI, Figs. 5, 13; Plate VII, Fig. 1). These bodies contained many refractive granules resembling fat granules, which at times show Brunonian movements, and a number of finer dark granulations. At times one or more large vacuoles can be seen within the protoplasm.

These bodies may be stained while fresh by adding a small amount of an aniline dye to the salt solution. If the precaution be taken to tease out carefully a dry bit of a colony on a cover-slip or slide, and the specimen be then diluted with a drop of salt solution and dried in the air or high above the flame, the organisms can be stained as are ordinary bacteria.

Treated thus with Loeffler's methylene blue or carbolfuchsin, the membrane stains deeply while the protoplasm takes on a lighter stain. The large pseudo-fat granules do not retain the dye, while, on the other hand, the small granulations stain more intensely than any other portion of the cell. At times the finely granular protoplasm does not completely fill the cell, but is separated from the membrane by a clear faintly staining hyaline area. These bodies remain unchanged in pure glacial acetic acid, and in 25 and 50 per cent. hydrochloric acid, but in pure hydrochloric acid they disappear.

Attempts were made to ascertain the chemical nature of the large granules, but they gave no reaction when treated with iodine, and hot

Millon's reagent and ether, although applied twelve times in succession, failed to dissolve them. The membrane also failed to give the cellulose reaction, but the fine black granulations would appear to be albuminous, since on boiling fresh specimens with Millon's reagent they often appear in the protoplasm as brownish-red particles.

The first colonies which were obtained from the original pus cultures consisted almost entirely of budding forms of the parasite (Plate VI, Figs. 4, 5, 6, 7, 17, 18, 19).

Mode of Growth.—The development of the parasite was then studied by means of small particles of the growth introduced into a hanging drop culture of gelatine and then allowed to grow (Plate VI).

From the round or oval bodies there occurs a growth of many long branching threads like mycelium, which are divided into shorter hyphæ by intervening fine transverse lines (Plate VI, Fig. 12). The protoplasm of the hyphæ is at first clear (Plate VI, Figs. 17, 18), but later it becomes beset with numerous fine granules or larger refractive bodies resembling oil drops (Plate VI, Fig. 3). In old cultures the protoplasm seems to arrange itself into long or oval hyaline bodies separated one from the other by a clear interval.

The numerous drawings of Plate VI represent many of the varieties which were found in the hanging drop cultures.

After several days, at various points along the sides of the hyphæ a number of knob-like projections (Plate VI, Fig. 15) of the limiting membrane may be seen, which gradually enlarge and at last form full-grown, round cells, or conidia, joined to the mycelium by means of their stems or sterigmata (Figs. 14, 15). These conidia may possess a double contoured membrane, together with fine or coarse granules. They may also become detached from the hyphæ, when they can either repeat the cycle of development just described, or increase by the process of budding and the formation of daughter cells. This latter phenomenon is of interest, since it is the only evidence of increase to be found in the tissues. The growth of the cultures is always accompanied by the formation of envelope and small crystals of oxalate of lime (Plate VI, Fig. 22).

Occasionally, in about the third generation of various cultures, certain colonies appeared to consist chiefly of the budding variety, only short hyphæ being observed here and there (Plate VI, Figs. 17, 18). In old cultures there appeared at times a mycelium which was finer in structure than the usual form observed.

Appearance of Cultures.—The organisms grow best on glycerine agar and potato, and develop slowly. In about seven days numerous fine grayish-white colonies appear on the surface, which later become pure white

and show minute prickles, thus resembling a number of minute white chestnut burrs scattered over the medium. They can attain the size of a split pea; they grow about two millimetres above the surface, and, if numerous, form a confluent membrane, suggesting a portion of the skin of a white mouse stretched over the surface of the medium. The growth is always firmly adherent to its substratum, and portions of the latter material are often removed with the colonies.

After the cultures have been passed through several generations of animals the loss of the mycelium causes these masses to appear as moist, flat gelatinous drops spread over the surface of the medium. In clear fluid cultures, for example, with bouillon, the parasite forms a thick interlacing meshwork through the medium, and no individual bodies can be found in the culture fluid.

In plate agar cultures the colonies show under a low magnifying power a granular centre from whose periphery fine ramifying branches grow out.

Colonies also develop readily on plain nutrient gelatine, 20 per cent. beer-wort gelatine, plain agar, 5 per cent. beer-wort agar, bouillon, fluid beer-wort (unfermented beer), Dunham's solution, milk, agar made from infusions of the various organs, Loeffler's blood-serum mixture, and human blood serum. Milk is not coagulated nor acidulated; no indol forms in Dunham's solution, and gelatine is not liquefied. No alcoholic fermentation occurred, nor was the formation of any gas observed after several weeks growth in the fermentation tubes containing glucose, lactose and saccharose bouillon.

Although cultures have been left for several months on apparently dried-up tubes, yet when transferred at the end of this time they grew luxuriantly, and in one case even proved pathogenic after this length of time.

The parasite will not grow as a strict anaerobe. This was proven by making several stab cultures into glycerine agar and covering the surface with melted gelatine. This tube was then placed in a Buchner jar with pyrogallic acid and KOH, and allowed to remain for one month at room temperature. At the end of this time the bodies introduced showed no evidence of increase.

The organisms develop in about one month when subjected to a temperature but slightly above freezing, and grow most favorably at a temperature ranging from 18-36° C. Their maximum temperature for growth is 40° C.

INOCULATION OF ANIMALS.

Fresh bits of tissue were introduced subcutaneously into a white mouse and a guinea-pig. The mouse died in 24 hours of a pneumo-

coccus septicæmia. The guinea-pig showed no change although observed for several months. Another guinea-pig was inoculated in the peritoneal cavity with diseased tissue after a laparotomy. After two months the animal was killed, but showed no pathological changes, with the exception of a few necrotic areas in the liver. Cultures, cover-slips and hardened specimens failed to reveal the presence of any parasites or bacteria in the liver, and cultures from the heart's blood and other viscera were negative.

These inoculations are only of negative importance, as they enable us, in conjunction with the facts mentioned above, to exclude tuberculosis.

A number of inoculations were made also with pure cultures of the organism. Four white mice were inoculated at the root of the tail. They survived the inoculation one month and were killed at the end of this time; the autopsy and cultures were negative. Two guinea-pigs inoculated subcutaneously with teased bits of cultures failed to show any reaction during two months, and several similar animals injected hypodermically with teased particles in bouillon gave negative results.

Several attempts were also made to reproduce the disease by implanting pure cultures upon scarified areas, or introducing them into small subcutaneous pockets made in the skin of a dog. These inoculations resulted only in abscesses containing the ordinary pus organisms. The organism was also introduced into the circulation of a rabbit, but when the animal was killed, two months later, the autopsy showed nothing remarkable, and stained hardened specimens of the lung showed these to be normal.

The following inoculation experiments, however, proved successful:

Exp. 1. Dog 1. The neck of a small dog was shaved and cleaned; the external jugular vein was exposed by Dr. Flexner, and one cubic centimetre of a suspension of teased colonies from a growth from the original pus was injected in the direction of the circulation. During the next two months the animal became somewhat emaciated and developed a discharge from the nose; at the end of that time it was killed.

Autopsy. Left external jugular vein obliterated and changed to a fibrous cord.

Lungs. Upon opening the thoracic cavity, both lungs presented a striking picture. Projecting from the entire pleural surfaces of both lungs there were numerous, generally discrete, pea-sized or larger, firm, light yellow nodules, which on section extend for the distance of from 0.5 to 2 centimetres into the lung substance. These areas are yellow, but are dotted here and there with lighter grayish-yellow apparently softer spots, half a pin-head in size. These nodules are round, their

peripheries are sharply marked off from the surrounding healthy lung tissue, and they are of about the consistence of a fresh pea. On section of both lungs numerous similar bodies were found scattered over the surface, but no cavities or caseous areas were seen. The bronchial glands were enlarged, firm, light yellow on section. The other viscera presented nothing of interest.

Bits of the pulmonary nodules and bronchial glands were teased finely and mounted in equal parts of 5 per cent. KOH and glycerine. A large number of the organisms were apparent in both specimens as highly refractive bodies with a well marked doubly contoured membrane; numerous budding varieties were also found. Fresh sections were placed in 10 per cent. formalin, followed by alcohol, and then stained with Stirling's gentian violet solution and decolorized with acetic acid 1-1000 and oil of cloves (Flexner's method). The nodules were then seen to consist of large central areas of necrosis containing an occasional giant cell and many fat drops, and surrounded by a zone of large epithelioid and lymphoid cells. The parasites are for the most part scattered throughout the necrotic area. The membrane stains dark blue, and the protoplasm a lighter blue, but no further differentiation can be made out. From the study of these bodies in fresh sections it is quite evident that there has been an extraordinary increase of them within the lungs, as from 6 to 10 spores often form the nucleus of an entire colony, and not a great number of these colonies were originally injected. No mycelium was present in the tissues.

Cultures.—The surface of the lung nodules was sterilized and relatively large portions were then dissected out with sterile instruments and placed on the surface of tubes of human blood-serum, glycerine agar, and beer-wort agar. In one week typical and pure growths occurred from the edges of the portions of tissue introduced, which consisted of bodies morphologically identical with those described above, and which later formed a profuse mycelium as in the cultures from the original case.

Pathological Histology. The smaller nodules scattered through the lung tissue consist of large accumulations of round, oval or elongated epithelioid cells and small round lymphoid cells, together with a moderate number of polynuclear leucocytes. These latter often accumulate around or apparently even invade the interior of some of the parasites which are probably dead. The larger and older nodules have undergone coagulative necrosis and in places contain groups of polynuclear leucocytes. Nodules stained for fibrin and glycogen gave negative results. Sections of pieces hardened in Flemming's solution show numerous large or small black fat drops for the most part within the caseous areas, but at

times they are seen in younger nodules, or in large *Staubzellen* on the edges of the diseased tissue. The younger nodules contain a few giant cells which often include a parasite.

The bronchial glands consist of a mass of confluent pseudo-tubercles intermingled with smaller areas of normal lymphoid tissue. These tubercles contain a number of giant cells, leucocytes and parasites, but show very little necrosis.

Both in the connective tissue of the larger bronchi and in the thick fibrous trabeculæ of the bronchial glands there are a number of mast-cells present.

Exp. 2. Dog 2. The next animal inoculation was made by injecting about two cubic centimetres of a suspension in sterile bouillon made from portions of the teased nodules of the lung of Dog 1 into the external jugular vein of a second dog.

In about two weeks the animal developed a sniffling cough and showed a well marked secretion of pus from the nose. This condition lasted several weeks, but, although the pus was examined microscopically several times, only one body having some resemblance to the parasite was found.

The animal was killed at the end of two months, and the autopsy showed each lung to contain about twelve firm, grayish-yellow, sharply circumscribed, round nodules, varying in size from a pea to a sparrow's egg, with an average diameter of from one to two centimetres. They are apparent as slight elevations over the surface of the pleuræ, and also as circumscribed nodules throughout the lung tissue.

The gross appearance of these nodules in the lungs of both dogs much resembles that of metastatic carcinoma. Nothing else of interest was noticed macroscopically.

The lung nodules from Dog 2, when teased and mounted in salt solution, showed a number of round or oval doubly contoured bodies exactly resembling those obtained from the lung of Dog. 1. The bronchial glands contained no organisms.

Pure cultures of the blastomyces were obtained by spreading large portions of the lung nodules over the surface of beer-wort agar.

On microscopic examination these nodules did not show such marked necrotic areas as were observed in the lungs of the first dog, but they here and there exhibited a tubercular formation with giant cells, epithelioid cells and lymphoid cells. It was not an uncommon thing to find a single adult organism apparently situated in the centre of an alveolus, which was filled up with a large collection of epithelioid cells, with a number of polynuclear leucocytes directly surrounding the parasites (Fig. 2, Plate VIII). This picture shows very closely what was sometimes seen in the

tissues of the original case. The general appearances were those of a chronic inflammation, and one would not be likely to regard the lesions as those of a neoplasm. Organisms of the budding form, without mycelium, were found in all the nodules, but not so many apparently dead forms were seen as in the first dog's lung. Giant cells were not numerous; most of those seen contained one or more parasites.

At the periphery of the younger nodules the alveoli showed marked swelling and projection of the epithelial cells, and often in the centre of an alveolus completely filled with epithelial cells one or more of the parasites could be made out. It would appear that this proliferation forms the beginning of the pathological process.

Exp. 3. A guinea-pig was inoculated with a suspension made from the lung nodule from Dog 2 into the peritoneal cavity, but an autopsy two months later showed no pathological lesions.

Exp. 4. Dog 3. A third dog received also an intravenous injection of about two cubic centimetres of a bouillon suspension from the lung of Dog 2, and at the autopsy two months later the lungs were seen to contain a moderate number of small, grayish pseudo-tubercles, the size of a pin-head. These nodules when teased in salt solution were seen to contain a few of the parasites and many fatty endothelial cells. The lesions were less marked and fewer than in the two previous dogs, showing that the organism was apparently losing its virulence.

Exp. 5. A guinea-pig was inoculated in the peritoneal cavity with some finely teased nodules from the lung of Dog 1. In about two weeks the epididymis on both sides became tense, swollen and painful, and later these swellings opened upon the skin surface and discharged a large amount of pus. This, when examined under the microscope, was found to contain a number of large doubly contoured bodies, many of which possessed small budding knobs and daughter cells. The formation of these knobs was also beautifully shown, and consisted in the rupture of the thick, doubly contoured membrane, and the protrusion through this opening of the protoplasm of the cell surrounded by the thin endosporium. These knobs later became invested by an outer membrane, and daughter cells were thus formed. Cover-slips stained for pus cocci gave negative results. The cultures were not contaminated.

These abscesses gradually healed, and at the autopsy, one month after inoculation, nothing was found except the fibrous scars of the pus cavities.

Exp. 6. Sheep. The external jugular vein of a sheep was exposed and 2 cc. of a suspension in bouillon of several fine colonies from a pure culture of the organism derived from Dog 1 were injected into the vein

by a sterilized hypodermic syringe. The needle was simply withdrawn and no ligatures were used.

From time to time a thin purulent secretion from the nostrils was noticed, but no parasites were found in the pus. In about five weeks the animal was killed, and both lungs contained a number of gray, semi-translucent, firm nodules, varying in size from a pin-head to a split pea. These nodules contained numbers of doubly contoured bodies, and a pure culture of the organism was obtained on beer-wort agar. Surrounding the nodules was quite a marked belt of young fibrous tissue.

Exp. 7. *Horse.* Many fine colonies from beer-wort agar culture from the lung nodules of Dog 1 were also teased and suspended in bouillon, and 2 cc. of this fluid were injected into the right external jugular vein of a horse, while at the same time a similar quantity was introduced into the subcutaneous tissues of the neck near the seat of the former injection.

In about thirty days there appeared an indurated abscess, the size of an egg, which soon opened superficially, and discharged a thick, creamy, yellow pus. This abscess was situated about midway between the angle of the jaw and the shoulder, on the right side of the neck, just beneath the seat of local inoculation. The skin over this abscess was shaved and sterilized, the abscess was thoroughly opened, and about 10 cc. of pus was collected in a sterile Petri dish. The opening closed in one week.

Cover-slips from this pus showed that it contained large numbers of doubly contoured refractive parasites, together with many eosinophiles, while cultures on beer-wort agar in one week showed a number of elevated gray, irregular membrane-like colonies. These consisted of doubly contoured blastomycetes, developing hyphæ as in cultures previously described. Plate VII represents a number of the varieties which were found in a teased colony and their method of growth. The tendency of the organism to develop by budding was again more pronounced at first, as in the cultures from the pus obtained from the original case. Although several cultures were made, none of the ordinary pus organisms developed, and cover-slips from pus also failed to reveal any such organisms.

At the end of two months the horse was killed and, although the superficial opening of the abscess had healed, yet in the deeper tissues of the neck a thick fibrous capsule was found surrounding a mass of thick yellow pus, which contained numbers of the blastomycetes.

On opening the thorax the pleural surfaces of both lungs showed a moderate number of firm, yellowish, sharply circumscribed nodules, varying in size from a split pea to an almond. Some nodules contained a central pus cavity, while from others pus could be squeezed as from a

sponge. These nodules were seen throughout the cut surface of both lungs. Neither the bronchial nor the cervical glands were affected, and the rest of the organs were normal.

The cultures from the pulmonary nodules became contaminated, but cover-slips from teased nodules contained typical blastomycetes.

Exp. 8. A teased nodule was put into the peritoneal cavity of a guinea-pig and the animal was killed in two months. Just beneath the point at which the tissue was introduced, between a loop of intestine posteriorly and the abdominal wall anteriorly, there were many organisms similar to those described above. It was impossible to take any cultures.

Exp. 9. A young pig (not guinea-pig) and several mice were inoculated subcutaneously with the pus from the abscess of the horse, but with negative results.

Exp. 10. A number of teased colonies from a beer-wort agar culture from the pus from the horse's neck were introduced into the peritoneal cavity of a guinea-pig, and in two months the animal was killed. On opening the abdomen a pea-sized, ovoid, yellow nodule was found enclosed within folds of peritoneum and attached to the testicle by a fibrous, long, thin pedicle. This mass consisted of a thin capsule, including a mass of soft semi-caseous pus, which contained numerous doubly contoured, refractive bodies. Cultures from the pus on blood-serum showed a pure growth of the parasite.

In addition, an attempt was made to produce a lesion in the liver of a guinea-pig by injecting a teased culture suspended in bouillon from the lung of Dog 1 into the mesenteric vein. At the autopsy about eight weeks later the liver was found to be normal, but at the point of entrance of the hypodermic needle a small abscess had developed. This was hardened in alcohol, and when stained was seen to consist of numerous pus cells and a large number of parasites, including many budding forms. Although the pus from the horse's neck was injected subcutaneously into one guinea-pig, and introduced into the peritoneal cavity of a second animal, the results in both cases proved negative.

Speaking generally, the effects produced on animals were the formation of typical pseudo-tubercles with caseation in the lungs of the first dog; in the second dog the necrosis was not so pronounced, although there was some slight caseation, and in the third dog the lesions were less marked and presented more the characters of foci of chronic inflammation. In the horse a local abscess was formed in the neck at the seat of inoculation and pseudo-tubercular nodules were formed in

both lungs. In the sheep the pulmonary lesions were also of a pseudo-tubercular character. In the three guinea-pigs which were successfully inoculated, encysted abscesses were the result, and a much larger number of organisms were contained in them than were injected.

The parasitic organisms in all the lesions presented the same general features and appeared identical in character with those in the patient. No mycelium was ever found in any of the lesions, although such developed in the cultures.

Many staining methods were tried on the diseased tissues from the animals, but the organism could be distinguished and studied well with the ordinary hæmatoxylin and eosin stain if the sections were thin.

If overstained with the hæmatoxylin the parasites would take up this stain, but if the eosin was used in excess they assumed the latter stain by preference. With the carbolfuchsin and aqueous methylene blue, the organism, particularly in the nodules from the lungs, could be made to acquire a double stain, the capsule being red and the protoplasm blue. The same effect could also be produced with eosin and aqueous methylene blue. Although safranin was tried and the organisms took up the stain well, they were less easily found among the surrounding stained cells. Flexner's method of staining with Stirling's gentian violet and decolorizing with acetic acid caused the parasite to stand out very prominently, but did not differentiate the contents of the organism.

CLASSIFICATION.

In our preliminary communication we classed our organism as an oidium for the following reasons: the organism did not ferment glucose, saccharose or lactose, and, although it only developed by gemmation in all the tissues, human and animal, yet upon artificial culture media it developed mycelia with the formation of conidia. After consulting Brefeld's writings, and also after a personal conference with Dr. Erwin Smith of Washington, an acknowledged authority in mycology, we were inclined to consider the parasite as an oidium. On further investigation we find that mycologists are not at all certain about the classification of either the yeast fungi or the oidia. We

find that there are a number of wild yeasts which do not ferment sugar and which form mycelium on the usual bacteriological media. In accordance with the prevailing custom in the nomenclature of organisms presenting characters similar to ours, we now prefer to designate the parasite as a blastomyces rather than an oidium.

COMPARISON OF OUR ORGANISM WITH THOSE OF PREVIOUS OBSERVERS.

In Busse's case the primary lesion was thought at first to be a sarcoma and the internal lesions resembled those of chronic pyæmia. Gilchrist's case resembled chronic scrofuloderma of the skin, wherein it resembled one variety of the cutaneous lesions of the present case. Corselli and Frisco described their case as one of sarcoma of the mesenteric glands, and Curtis speaks of his case as a tumor of myxomatous appearance of the thigh and loin.

The lesions in our patient differ then from those in all the previous cases with the exception of Gilchrist's case of blastomycetic dermatitis, in that ours simulated lupus vulgaris, and in the inoculated animals presented lesions somewhat resembling tuberculosis with acute and chronic inflammation. Our organism was also distinctly pyogenic both in the original case and in inoculated animals.

The organism in our case, although apparently differing but little from those described in previous human cases, is markedly distinct in its method of growth on media by profuse mycelium and conidia. In all the other cases the growth has been described as being chiefly by gemmation. In her experimental work on blastomycetes Rabinowitsch demonstrated that a number of wild yeasts which were pathogenic in animals did not ferment sugar and formed mycelium. Whether all these parasites have the power of producing different pathogenic pictures according to the organism which they attack, or whether they are varieties of the same species, is a question which is very difficult of solution in the present crude state of our knowledge regarding this class of micro-organisms.

COMPARISON OF OUR ORGANISM WITH THE SO-CALLED CANCER PARASITES.

As we have already mentioned, many of the investigators who have reported cases where blastomycetes were believed to be the cause

of pathogenic lesions, have made the assertion that the bodies present in cancerous tissues, which some previous observers had wrongly described as protozoan parasites, are really blastomycetes. Not a few authors have also described many of the lesions which have been produced in animals by inoculation with blastomycetes, as genuine neoplasms, and have adduced these in support of the parasitic origin of malignant tumors. Sanfelice, especially, described the lesions produced in the mammary glands of a bitch and in the comb of a cock as presenting a carcinomatous appearance. Sanfelice, Roncali, Kahane, Binaghi and others have also asserted that the curious bodies which are often found in sarcomatous and epitheliomatous tissues are really blastomycetes, although they have failed to obtain them in cultures and have not succeeded with inoculation experiments. One of us (Gilchrist) has already made comparison between the blastomycetes which he found in his case and the so-called parasites in twenty-three cases of epithelioma of the skin, and demonstrated that there were very few points of resemblance between these forms. He concluded that the evidence appeared to be strongly against the idea that the bodies found in cancer are blastomycetes. We have compared our parasites in this case again with the so-called cancer parasites, and feel still more strongly in favor of the view that the bodies found in cancer are really the result of epithelial degeneration. The same arguments can be presented here against the view that the cancer bodies are blastomycetes, which one of us (Gilchrist *) advanced in regard to these bodies being considered as protozoa. These are mainly as follows: (1) The so-called cancer parasites found in twenty-three cases of cancer, five of which were of the lip, bear very little resemblance to the blastomycetes either of Gilchrist's case or the present one. (2) They are by no means present in every case of undoubted cancer. (3) They are often found in other diseases, *e. g.* in benign growths of the skin, in tuberculosis cutis, in syphilitic ulcers of the skin, and in other cases. (4) There is an entire absence of uniformity in the appearance of the cancer bodies, those described for one case being often entirely unlike those seen in another. (5) The descriptions given of developmental stages are

* *Johns Hopkins Hospital Reports*, i.

vague and by no means convincing. (6) Special stains and refined technique are required to bring out many of the so-called cancer parasites, whereas the blastomycetes are demonstrable by all methods and stain readily. (7) The blastomycetic dermatitis of Gilchrist's case and the present one do not approach the clinical or pathological characters of epithelioma, but bear more resemblance to tuberculosis of the skin. (8) No positive results have ever been obtained from the numerous inoculation experiments which have been made. (Roncali has isolated what he calls a blastomyces from an epithelioma, but one can often cultivate many kinds of fungi from superficial epitheliomata.) (9) The ready demonstration of the blastomycetes by liquor potassæ does not apply to the so-called cancer parasites.

The theory of the blastomycetic origin of malignant tumors has also received little support from experimentation. Shattock and Ballance made a large number of cultures from malignant tumors upon all of the various culture media, but with negative results. At the suggestion of Dr. Flexner we have also made cultures upon beer-wort agar (the most favorable medium for yeasts) from about ten sarcomata and carcinomata. Our method consisted in sterilizing by heat both sides of a small block of tumor immediately after removal, and then punching out a cylinder with a sterile trochar, teasing the tissue in a sterile Petri dish, and plating the finely divided tumor in beer-wort agar. The plates usually remained sterile, and although an occasional colony developed this never consisted of yeasts. The plates were observed for weeks. The theory of the blastomycetic origin of malignant tumors, we think, needs much more confirmatory evidence before it can be accepted.

TREATMENT.

We had intended to place the patient under the influence of ether and curette the cutaneous lesions very thoroughly, and then apply the nitrate of silver stick to the denuded surface. In view of the fact that the parasites were not very numerous and the course of the disease had shown itself to be exceedingly chronic, we considered we had good reasons for supposing that the disease could thus be easily cured, especially since the neighboring lymphatic glands were unaffected. But

the patient was called away from the hospital on account of the serious illness of his wife, and he has remained without treatment up to the present time. We received a letter from him recently, six months after he left the hospital, in which he says that his face and eyelids have almost healed up. The disease, all through its course, has shown a strong inclination to spontaneous cure. The prognosis, in our opinion, therefore, is good.

SUMMARY.

The case reported in this article is one of a somewhat extensive cutaneous disease, which occurred in a man, 33 years of age, who gave the following history: The disease first made its appearance, eleven and a half years ago, at the back of the left ear, as a pimple which soon became pustular. The process extended forward very slowly and gradually encroached upon and covered almost the entire face, the central portion of which now presents an atrophic cicatricial condition. Another similar lesion occurred, one month after the primary invasion, on the back of the hand, which healed in about four years, after treatment with caustic. A third lesion appeared on the right side of the scrotum (six months after), which increased in size for a year and then healed spontaneously. A fourth inoculation appeared on the anterior surface of the left thigh just above the internal condyle, and grew for a year, after which it gradually healed spontaneously. A fifth lesion appeared on the back of the neck and also healed spontaneously after growing for a year. The disease when first examined presented many of the features of a lupus vulgaris.

There were no enlarged lymphatic glands and the patient's health had always been good. The family and personal history revealed no syphilitic or tuberculous taint.

Sections from the cutaneous lesions showed the presence of what appeared to be budding blastomycetes. The sections also presented pathological features similar to those seen in the first case recorded by Gilchrist; in many sections almost typical tubercles were found.

The organisms in the tissue are chiefly spherical, unicellular bodies varying from 10-20 μ in diameter, and consist of a doubly contoured membrane, which encloses a fine granular protoplasm with sometimes

a vacuole. Many budding forms in various stages were found; no nucleus could be demonstrated, neither were any mycelium or hyphæ present in the tissues. The parasites were almost always found outside of cells, comparatively few being enclosed in giant cells.

Pure cultures of the organism were obtained directly from the cutaneous lesions in two places from the pus squeezed out from between the papillomatous variety of the lesion. The organism grew on all ordinary media, and especially well on potato and beer-wort agar. The cultures showed both budding forms and a fairly profuse mycelium. Older cultures, carried through many generations, produced sometimes little or no mycelium. The organisms in the cultures were round, ovoid, doubly contoured, refractive bodies, varying in size from about 10 to 20μ in diameter. The mycelium was, on rare occasions, of two varieties, very fine and also coarse with sessile buds and conidia.

Dogs, a horse, a sheep and guinea-pigs were successfully inoculated, the most striking results being nodules, grossly simulating tumors, in the lungs. Microscopically these nodules were of a chronic inflammatory nature and contained numerous parasites identical in appearance with those in the patient. In the tissues of none of the animals successfully inoculated was any mycelium found. Since our organism did not ferment sugar and produced in cultures mycelium, it may either belong to the blastomycetes or to the oidia, but in conformity with prevailing nomenclature we regard it as a blastomyces.

We shall term the disease which has been produced by this organism *Blastomycetic Dermatitis.* We give the name *Blastomyces dermatitidis* to the parasite which we have isolated and described in this paper.

In closing we are of the opinion that it would be advisable to examine more carefully all tuberculous lesions of the skin, and especially those of tuberculosis verrucosa cutis, for the presence of blastomycetes. This can be readily and rapidly done by soaking the unstained sections in ordinary liquor potassæ, when the organisms if present will stand out as doubly contoured refractive bodies.

DESCRIPTION OF PLATES IV–VIII.

PLATE IV.

Photograph of patient showing the cutaneous lesions of blastomycetic dermatitis.

PLATE V.

Fig. 1 represents a microscopic section of blastomycetic dermatitis. The epidermis (*e*) is hypertrophied and encloses numerous miliary abscesses (*a*), which contain the parasitic organisms (*p*). In the corium (*c*) miliary abscesses (*b*) are also present as well as pseudo-tubercles (*n*), which enclose parasites (*p*). The corium is filled with masses of granulation-tissue cells and contains a few scattered giant cells (*G*).

Figs. 2-16 represent various forms of the blastomycetic parasite as observed in the tissues of the patient. Various stages of the budding process are shown. The refractive double contours of the enveloping membrane and the protoplasmic contents of the parasite are depicted. The organisms drawn in Figs. 8 and 9 appeared to have an outer irregular fibrous coating. Fig. 16 shows a group of parasites in the corium.

PLATE VI.

Figs. 1-21 represent various growth-forms as observed in a colony of the blastomyces cultivated in a hanging drop of gelatine on the slide. Figs. 6, 7, 10, budding yeast forms. Figs. 16, 17, 18, beginning development of hyphæ. Other figures show various mycelial developmental forms with conidia. Fig. 22, crystal of calcium oxalate.

PLATE VII.

Figs. 1-16 represent a number of varieties of the developmental stages of the blastomyces as obtained from a colony from the pus produced in the horse's neck. Fig. 1 is a single round organism with a large clear space in the centre. Direct budding forms are seen in Figs. 2, 4, 14 and 16. A sessile bud on mycelium is shown in Fig. 8. Mycelial threads forming from the organism are seen in Fig. 3, whereas in others the mycelium shows attempts at forming the round or oval form directly in the axis.

PLATE VIII.

Fig. 1 shows a small miliary abscess situated in the epidermis. It contains two parasites, a number of polynuclear leucocytes, nuclear fragments, and nuclei of detached epithelial cells.

Fig. 2 represents a portion of one of the most recently formed nodules from the lung of the first dog inoculated. The organism is in the centre, a number of polynuclear leucocytes lie between the parasite and the epithelioid cells, and lymphoid cells constitute the peripheral portion.

Figs. 3, 4 and 5 show giant cells which were found in the deeper portion of the corium of the patient's cutaneous lesions. The vacuole in the parasite and the budding are well marked in Fig. 5.

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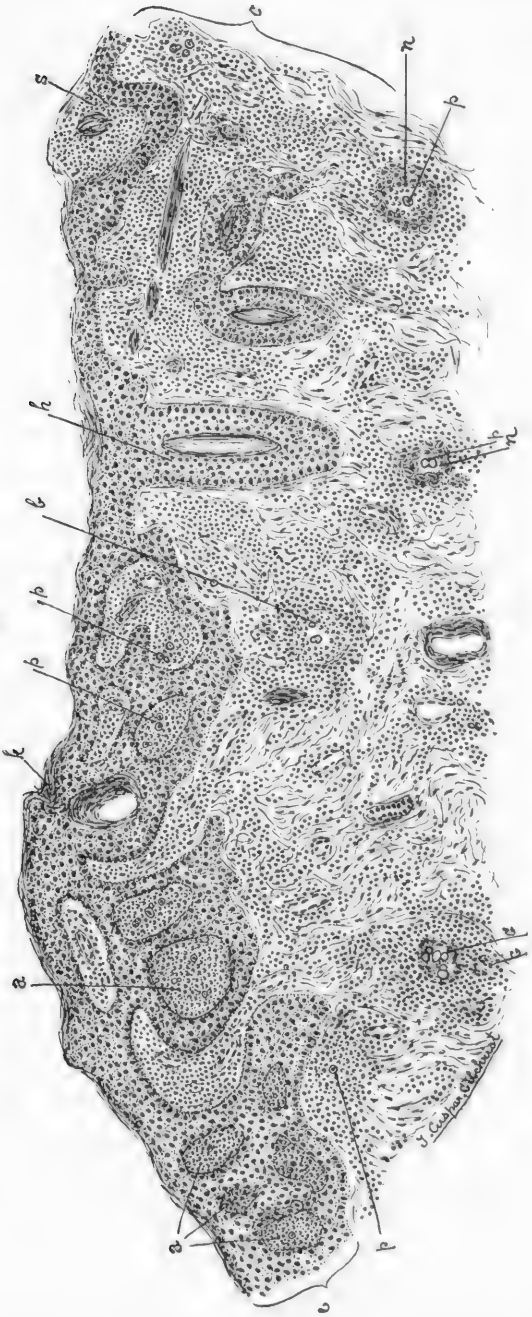
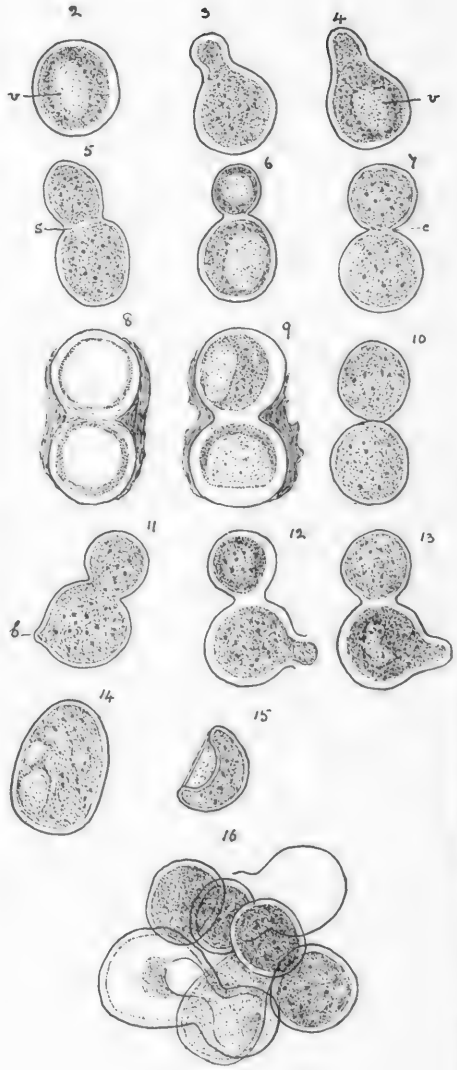
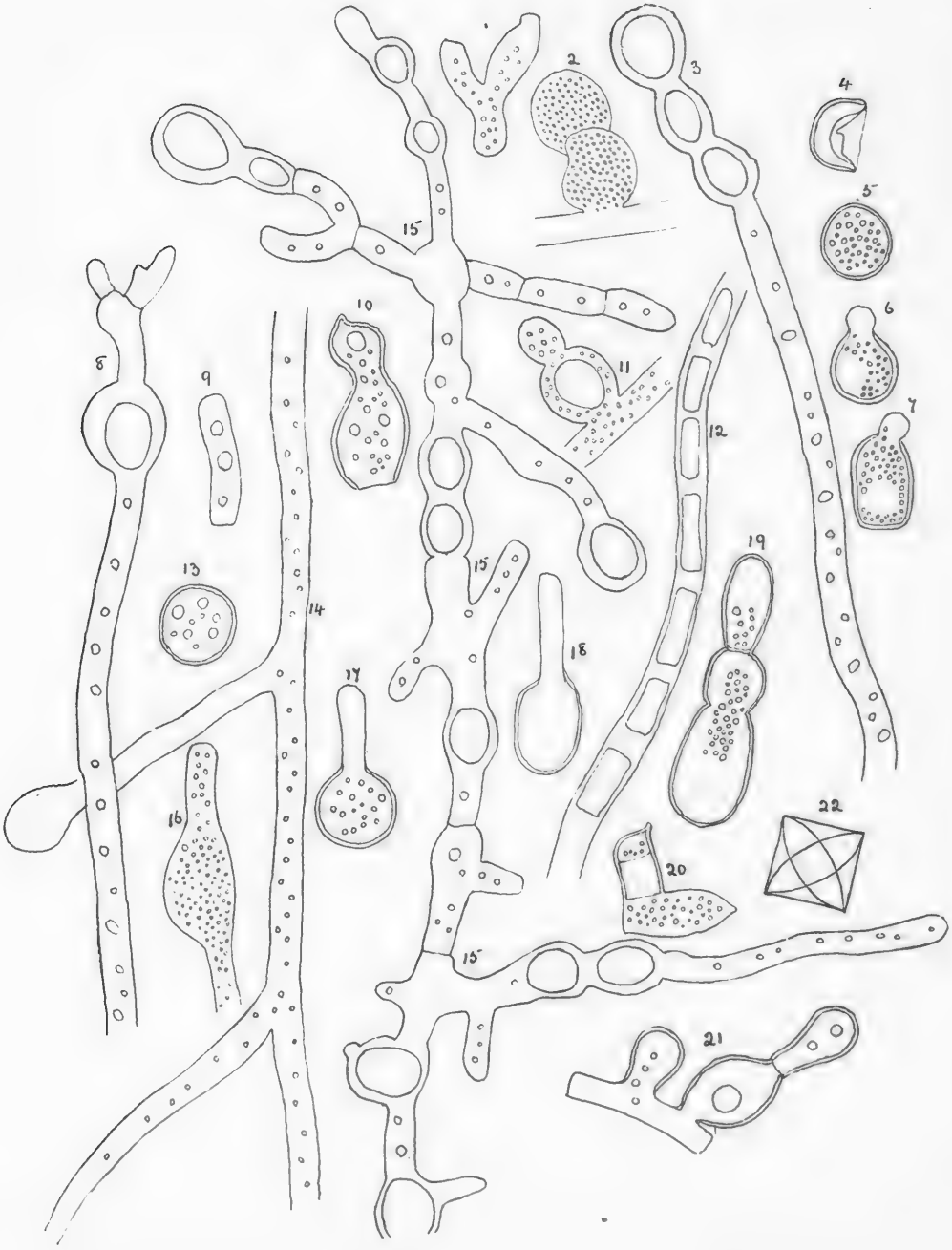


Fig. 1.

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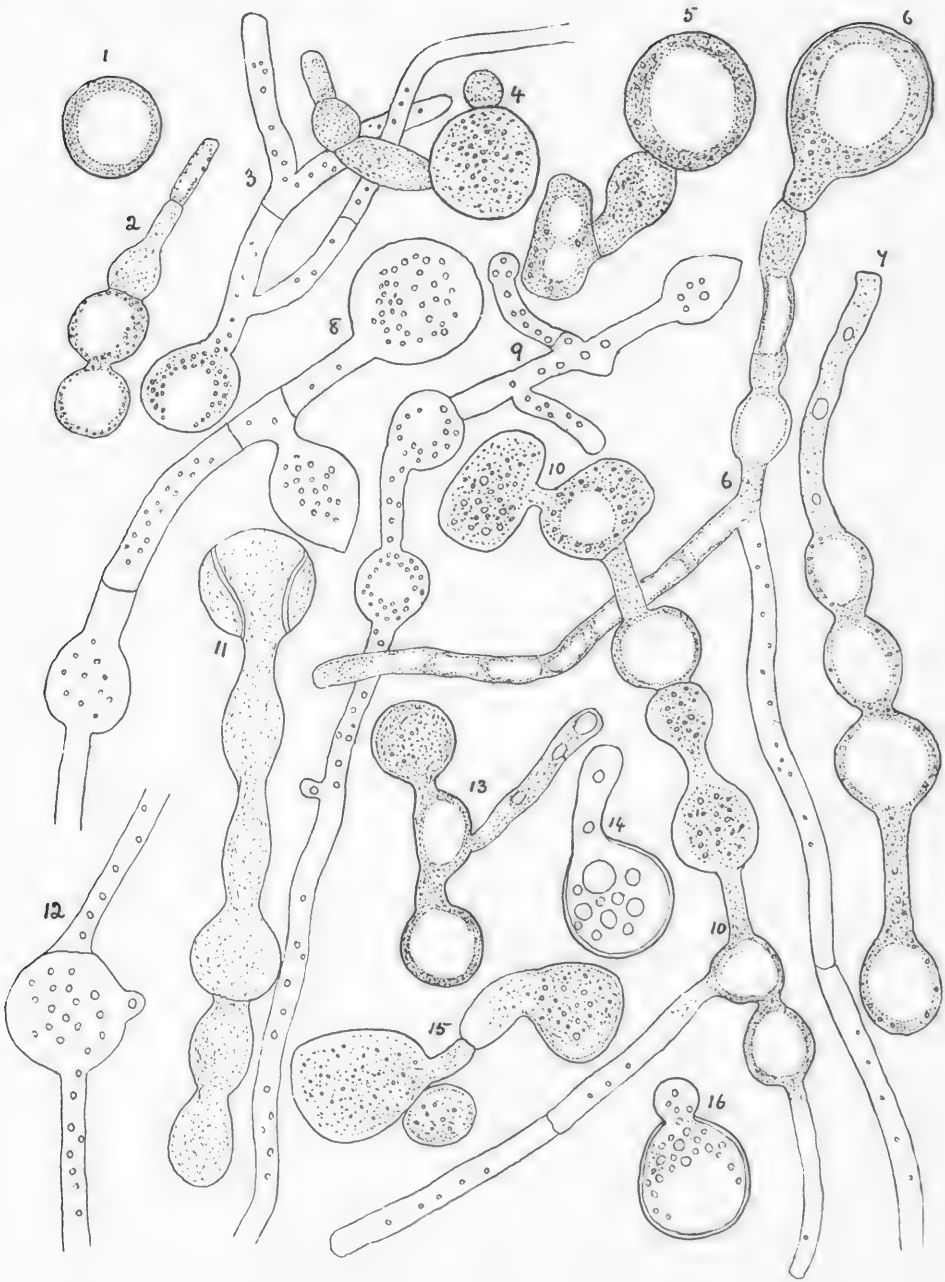
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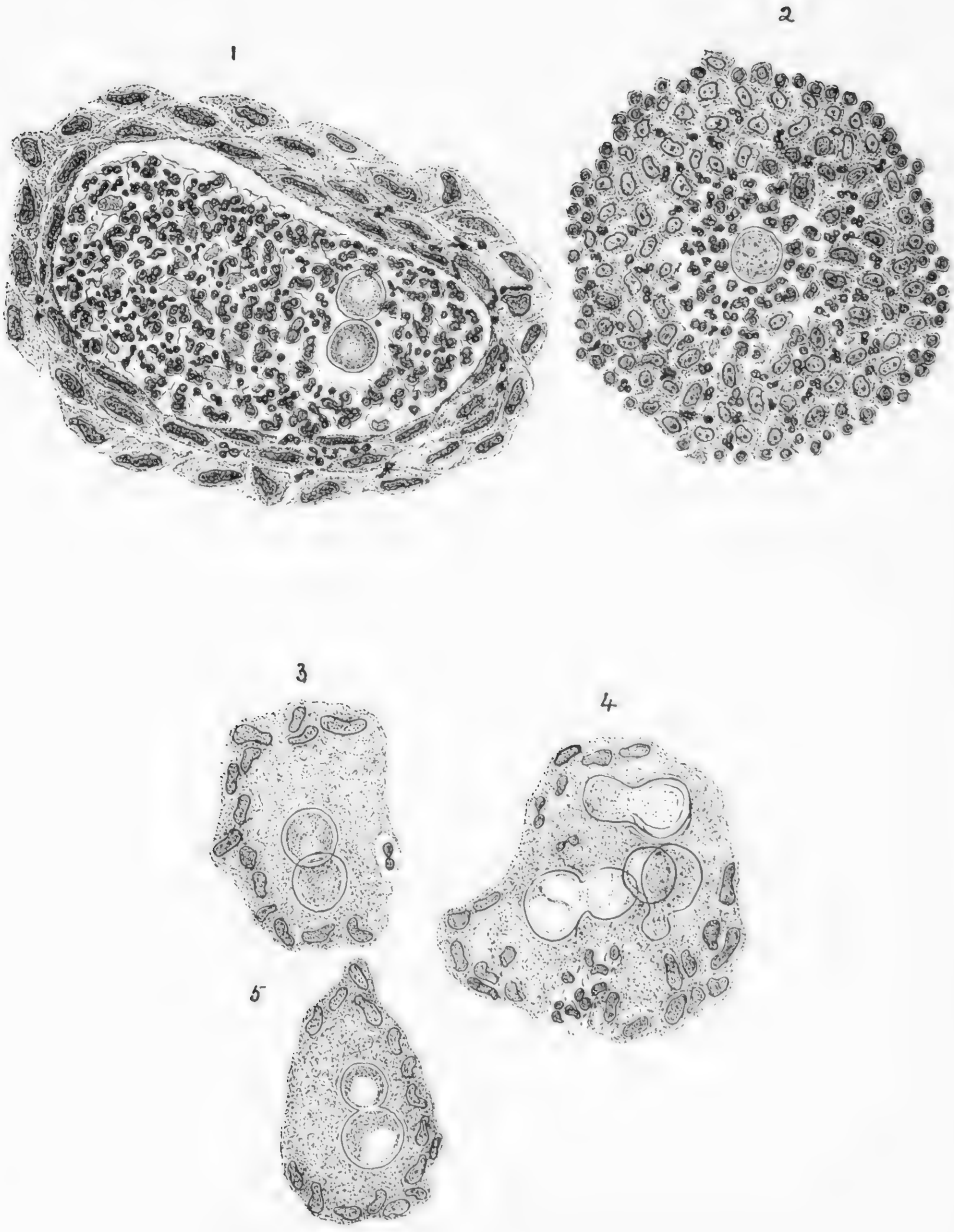
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ON THE HÆMOCYTOZOA OF BIRDS.

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PLATE IX.

Since the discovery of the hæmocytozoa of malarial fever considerable attention has been paid to parasites of a similar nature which have been found in various animals. Those which inhabit the red corpuscles of the blood of birds have excited particular interest because of their close resemblance to the malarial organisms. While numerous European observers, for the most part clinicians and pathologists rather than zoologists, have studied the parasites occurring in the blood of birds, no investigations have up to the present time been made in this country.* Dr. Thayer suggested to Mr. MacCallum and the author to make a study of such organisms as were to be found in the blood of birds captured in the neighborhood of Baltimore. We devoted a portion of the summer of 1896 to this work, which has been done in the Clinical Laboratory of the Johns Hopkins University and Hospital. To Dr. Thayer we are indebted for the many suggestions which he has given us.

History.—Almost ten years before the discovery of the malarial organism by Laveran in 1880, Ray Lankester † had observed in the blood of frogs very small worm-like bodies free in the plasma. Butschli,‡ in 1876, mentions the fact that he has frequently found in the red corpuscles of frogs, peculiar bodies occurring at the side of the nucleus; his figures show that these bodies represent an intracorpuscular stage in the life history of the organism which Lankester saw in the free state. This is apparently the first observation of a parasite within the red corpuscles —a hæmocytozoon.

* A brief preliminary report of our observations appeared in the proceedings of the Johns Hopkins Hospital Medical Society, *Bull. of the Johns Hopkins Hospital*, March, 1897, No. 72, p. 52.

† *Quarterly Jour. of the Microsc. Sciences*, xi (1871).

‡ *Abhandl. der Senkenberg. naturf. Gesellsch.*, 1876, p. 261.

Ignorant of these observations, Gaule * later described in the red corpuscles of the frog bodies which he speaks of as "Würmchen." These he believed to be formed from the protoplasm of the corpuscle as a result of degenerative changes; he gave them the name *Cytozoa*. Soon afterwards, however, Ray Lankester † established the parasitic nature of these structures and their identity with the organisms which he had previously observed.

Danilewsky ‡ (2) in 1885 described, for the first time, intracorpuscular parasites in the blood of lizards, tortoises and birds. The organisms which he has found in the bird differ markedly from those in cold-blooded animals—frogs, lizards, tortoises. Most conspicuous at first sight is the fact that the latter do not transform the hæmoglobin into pigment granules; other points of difference are, however, not wanting. We have to do at present only with the organisms found in the blood of birds, these most closely resembling the malarial parasites of human beings.

In the numerous articles which Danilewsky has written a variety of forms have been described:

As *pseudo-vacuoles* he describes bodies which appear as clear areas of variable size and shape within the red corpuscle; in the pseudo-vacuoles he finds black granules of pigment, a product of the transformation of the hæmoglobin.

As *pseudo-vermiculi* he describes elongated bodies with an active wriggling motion which are found free in the plasma. He observed two forms: one non-pigmented similar to the organisms found in cold-blooded animals; the other arising from pigmented pseudo-vacuoles which have escaped from red corpuscles.

To the pigmented flagellate organism, which he describes as a distinct species of parasite, he gives the name *Polymitus avium*. These develop as pseudo-vacuoles in the red corpuscles and subsequently undergo the process of flagellation.

At times the flagella become detached and maintain their active motion for a time; to these free flagella he has applied the term *pseudo-spirilla*.

Although Danilewsky had, for a long time, no opportunity of examining malarial blood, he was much impressed with the similarity between the organisms which he had observed and those which had been described by Laveran, and Marchiafava and Celli. The full-grown parasites,

* *Arch. f. Anat. u. Phys.*, Phys. Abth., 1880, pp. 57, 375; ibid., 1881, p. 297.

† *Quarterly Jour. of the Microsc. Sciences*, Jan. 1882, p. 53.

‡ References to literature at end of this article.

pseudo-vacuoles, in the birds examined by him were approximately half-moon-shaped bodies which suggested the crescents of irregular malarial fever; the flagellate forms differed but little from those found in man. In a short article, published in 1886 (4), he raises the question whether or not these organisms are identical, and answers it in the affirmative.

Grassi and Feletti (12) found, particularly in sparrows (*Passer hispaniolensis*) and in pigeons, what they regarded as two distinct types of organism. One form was the half-moon-shaped parasite already described by Danilewsky; this they believed to be closely related to the crescents of malarial fever. They established the genus *Laverania* to include the two forms to which they gave the specific names, *Laverania danilewskyi* (of the bird), *Laverania malariae* (of man).

The second type of parasite, described for the first time by them, was a smaller organism of irregular shape occupying one end of the corpuscle. This body they regarded as closely related to the amœboid forms of the malarial parasites; they established, therefore, the genus *Haemamoeba* to include them. They find in birds two common species of these small, irregular parasites, which they have termed the *Haemamoeba subpraecox* and *Haemamoeba relicta*. They have further described a third type of organism which they observed in a single bird; to this they gave the name *Haemamoeba immaculata*, owing to its freedom from pigment granules.

Celli and Sanfelice (1) have also attempted to bring the parasites found in the blood of birds into conformity with those of the malarial fevers. They base their classification of the parasites which they have observed upon the rapidity with which these organisms undergo their cycle of development. They find:

(1) Parasites with slow development, reproducing after eight days of growth. Morphologically these are the half-moon-shaped bodies of Danilewsky. Celli and Sanfelice think that they correspond to the quartan organism of man.

(2) Parasites with a shorter period of development.

(3) Parasites with rapid development.

Of the two latter varieties they could not determine the exact length of the cycle of development. Morphologically they are irregular bodies lying at one end of the corpuscle. They differ from one another, according to Celli and Sanfelice, in the size of the sporulating form, which in those with quick development is smaller and divides into fewer segments.

The second variety of parasite, that with a cycle of development of mean duration, they think, corresponds to the tertian organism; the third variety, that with rapid development, to the organism of æstivo-autumnal

fever. Although Celli and Sanfelice insist upon an analogy between these forms and those of man, they do not assert that they are identical.

They found one or more of these varieties of organism in various species of birds; two varieties may co-exist within the same host. In some instances, where they found for a number of days but one variety, there appeared later a second, which became eventually far more abundant and finally replaced the first. They are in fact inclined to believe that one form can change into another; that they are dealing with a single polymorphous parasite. They have never succeeded in transferring infection from one bird to another of a different species. Hence they come to the conclusion that despite the great morphological similarity between the parasites of different birds they are yet separate organisms, each species of bird having its own variety of parasite which will flourish in this species alone.

Danilewsky (7) has attempted to demonstrate the existence in infected birds of symptoms analogous to those of the malarial fevers. He describes an acute and a chronic "malarial" infection in birds. The acute condition is characterized by rise of temperature, loss of weight and general symptoms of illness. In the chronic infection there is no rise of temperature; after death, however, the spleen is found to be much enlarged and pigmented. The acute infection is caused, he thinks, by the small irregularly-shaped parasite described by Grassi and Feletti; the chronic by the half-moon-shaped bodies. In his later articles Danilewsky does not speak with definiteness of the identity of the birds' parasites with the malarial organisms of human beings; he maintains, however, that the varieties described are very similar if not identical.

Labbé (20), more recently, has carefully studied the intracorpuscular parasites of the blood of vertebrates from a purely zoological point of view. He classifies the various organisms that have been described by himself and previous observers according to structural peculiarities. In birds' blood he recognizes the two types distinguished by Grassi and Feletti. The names which he has assigned to them do not imply any necessary correspondence with the malarial parasites of man. To the small irregular forms he gives the generic name, *Proteosoma*. Of this genus he finds but a single species, *Proteosoma grasii*. To the elongated forms, which lie curved about one side of the nucleus of the corpuscle, he gives the name *Halteridium*. Of these also he finds but a single well-marked species, *Halteridium danilewskyi*.

A large number of inoculation experiments have been performed. Celli and Sanfelice, Laveran and Labbé claim to have successfully inoculated organisms from the blood of one bird into another of the same

species. Grassi and Feletti were never successful in such attempts. Di Mattei (11) has more recently performed a large number of inoculation experiments with pigeons. He injected by various methods blood of infected birds into those not infected. In 83 experiments he obtained universally negative results. He injected blood of malarial patients into 16 pigeons with no consequent infection. The same result was obtained in four instances in which he injected the blood of infected birds into the arm veins of healthy men. He concludes, therefore, that, though the parasites of birds morphologically resemble the organisms of malaria, they are essentially different.

PERSONAL EXPERIENCES.

Our studies were carried on in the months of June, July, August and September, 1896. One hundred and twenty-five birds were examined. Blood was drawn from the wing, and both fresh and dried and stained specimens were studied.

About one hundred birds were examined which had been captured in the neighborhood of Baltimore. Of these the greater number, eighty, were English sparrows (*Passer domesticus*); of the remainder, twelve were red-wing blackbirds (*Agelaius phoeniceus*). The others belonged to a variety of species. The great majority of these birds were obtained from places outside the city and near the Patapsco River or Chesapeake Bay, districts notoriously malarious. Of the eighty English sparrows twenty-one were caught in the city on the Hospital lawn; none of these showed infection. Of the remaining fifty-nine sparrows one in twelve was infected. These figures are almost too small to offer even a suggestion as to the influence of locality on the frequency of infection.

Among the red-wing blackbirds infection seems much more common; of twelve birds, six were infected.

In fifteen birds altogether the blood showed intracorpuscular parasites in varying abundance. Among these birds the following species were represented: *Passer domesticus*, *Agelaius phoeniceus*, *Melospiza georgiana*, *Melospiza fasciata*. It is evident that the observations were not made upon a sufficiently large scale to arrive at any very general conclusions as to the species prone to infection, or as to the local conditions favoring infection. Nevertheless, they indicate, prob-

ably with sufficient accuracy, the commonest varieties of parasites found in this locality.

While at his home in Dunnville, Ontario, not far from Toronto, Mr. MacCallum examined a number of birds. Of twenty-five, in five were found intracorpuscular parasites. At present there is little if any malarial fever in this locality, although at one time the disease was more common. Of two great-horned owls (*Bubo virginianus*) examined one was found to have a most severe infection. In four of five crows (*Corvus americanus*) the blood contained similar organisms. The birds whose blood was negative included a few individuals of a variety of species.

Types of Organism. Two varieties of organism may be distinguished among the intracorpuscular parasites which we have studied. These correspond to the two forms described by Grassi and Feletti and subsequent observers. Characteristic of each group is the morphology of the full-grown organism. In the one an irregularly shaped body, containing pigment granules, occupies one end of the red corpuscle, while the nucleus of the corpuscle is displaced from its normal central position into the opposite end. The other group is characterized by the fact that the full-grown parasite is an elongated pigmented body lying along one side of the normally situated nucleus and curving more or less over its two extremities; this is the so-called halter-shaped form—*Halteridium* of Labbé. Both develop from very small, non-pigmented, approximately spherical bodies.

In the first variety as the body grows it increases in size gradually in all dimensions, and in growing the parasite exerts sufficient force to cause the displacement of the nucleus. In the second, whether as a result wholly due to peculiarities of the organism or to forces acting within the corpuscle, such, for example, as resistance to the displacing of the nucleus, growth takes place more rapidly along that axis which corresponds with the long diameter of the corpuscle. We may distinguish, then, according to their method of growth, two forms, one irregular and the other elongate. This is their most conspicuous difference; other peculiarities of the two forms are, however, not lacking.

In the majority of infected birds either one or other form was

observed alone. Nevertheless, in three birds the two varieties were present at the same time. Hence the possibility suggests itself that the two forms may represent different phases in the life history of the one organism. This view, as we have mentioned, has been supported by Celli and Sanfelice. In birds where one or the other form is present it is, however, possible to trace the whole cycle of development from hyaline non-pigmented bodies to full-grown organisms; it cannot be doubted that the full-grown parasites of both forms segment to form new hyaline bodies. The cycle of development is very different in the two forms and leaves little ground for the belief that one form may appear as a stage in the development of the other. Moreover, in the human being where the multiplicity of species of the intracorpuscular parasites, though not absolutely undisputed, still rests upon much stronger evidence than does that of the organisms of birds, the possibility of an infection with two kinds of malarial parasites at the same time is well known. The frequency of such infection in this locality, for example, is shown by the fact that out of 542 cases of malarial fever studied by Thayer and Hewetson,* there were eleven instances of double infection. The three instances above mentioned, in which the two forms were simultaneously present in the same blood, occurred in blackbirds. Of the blackbirds which we examined one-half contained intracorpuscular parasites. It is not surprising, then, that, where infection is so common, combined infections should be more frequent. Among the English sparrows, of which only one in fifteen was infected, double infection was not observed. For these reasons, then, we believe the two forms to be distinct, and shall therefore consider them separately.

Irregular Parasite (*Proteosoma of Labbé*). The earliest phase of development of this organism is represented by an approximately round, clear body situated within the red corpuscle. Since the body is refractive and gives no indication of amœboid movement, it is hardly to be distinguished in the fresh blood from the vacuoles, which are not infrequently found in the red corpuscles. Nevertheless, in specimens of blood, dried, hardened and stained with eosin and methy-

* *The Johns Hopkins Hospital Reports*, Vol. V.

lene blue according to the method of Romanowsky, modified somewhat by Heppener,* the earliest stage of the parasite is clearly recognizable as a very small body of an oval, round or slightly irregular shape (Plate IX, Fig. 1). The way in which these structures stain is fairly constant; the peripheral portion takes a rather deep blue color, while within this blue rim the substance of the parasite is almost completely unstained.

As the parasite grows larger it acquires pigment, at first a fine black granule situated within the external staining portion of the organism (Plate IX, Figs. 2-5). As the parasite grows, other granules appear. These are, in the great majority of cases, collected into a more or less loose clump, situated as a rule near the periphery of the body; at times, indeed, one sees a particle of pigment lying apparently upon the surface of the parasite. In the neighborhood of the main clump there are usually one or more isolated granules. In the large forms these may sometimes upon careful observation be seen to have a slow motion away from or towards the main mass; they may merge into it, while the clump itself may slowly change its form, presumably by a rearrangement of the constitutent particles. This collection of the pigment into a single clump in the half- and full-grown forms is almost characteristic of the irregularly shaped organisms; at times, however, one sees parasites with discretely scattered pigment granules. The shape of these pigmented bodies is variable; short blunt processes are very frequent. Although we have carefully watched these organisms in fresh blood for a long time, both at room temperature and on the warm stage at 40-41° C., we have not been able to convince ourselves of any change in their shape. Nevertheless, the irregularity in the shape of the bodies and the frequency with which processes are seen, make it probable that a very slow amœboid movement does occur.

In stained specimens the distinction between the peripheral deeply stained rim and the central unstained portion is lost in these larger forms; the whole body takes a fairly deep color. The stain is, however, not uniform, the body having a somewhat granular appearance; we have not seen the definite granules of staining material which can

* *Med. Pribav. K. Morsk. Sborn.*, Jan. and Feb., i (1895), 67.

be made out in certain varieties of the elongate organism (p. 92). Often one may distinguish an approximately round, sharply marked off area, which is almost unstained at times. This may contain one or more faintly staining bodies. This structure, possibly always present in the larger forms, though obscured in some instances by a too deep stain, is what has been described as a vesicular nucleus containing nucleoli.

The growing organism, which at first may be situated in any part of the corpuscle, comes to occupy one or the other extremity. Not infrequently two parasites, or it may be more, are present in the same corpuscle. In such cases they are almost always of the same size; occasionally, however, one is much larger than the other. As the parasite increases in size a change takes place in the position of the nucleus of the corpuscle; it is deflected from its normal longitudinal and central position until it not infrequently comes to lie almost or quite at right angles to the long axis of the corpuscle and at the same time nearer one extremity.

One would suppose that the displacement of the nucleus was the mechanical effect of the growth of the parasite, and, in fact, in many cases the parasite fills the whole of one extremity of the corpuscle with one of its sides flattened out against the nucleus which lies crowded transversely into the other extremity. Not infrequently, however, as Labbé has pointed out, a small parasite may be at one end of the corpuscle, while the nucleus is displaced into a transverse position, although it is touched at no point by the parasite (Plate IX, Fig. 2). The explanation of the process in such cases does not seem, then, to lie altogether in a mechanical displacement of the nucleus, but apparently involves a consideration of the internal structure of the red corpuscle and of the forces that hold the nucleus in place. Upon these points, beyond the obvious fact that there is a marked disturbance of such forces, we can say nothing.

It is interesting in this connection, that one not infrequently may come across parasites surrounded by a rim of red blood corpuscle from which the nucleus is absent, having been apparently extruded (Plate IX, Figs. 6 and 7); this picture we have observed only with the variety

of organism now under consideration. In such cases the remains of the corpuscle are somewhat distorted; often one extremity is drawn out into a point. The stroma of the corpuscles containing the half- and full-grown forms is not decolorized, nor is any alteration of the nucleus to be seen.

With the irregular organism the whole process of segmentation, which is closely similar to that of the malarial parasites of man, takes place in the circulating blood. Bodies representing the first stages of the process are at times met with in great abundance. The parasite assumes an approximately round form; the pigment collects into a solid block which occupies the centre of the body. The outline of the parasite is not perfectly even, but shows more markedly, in proportion as the process has advanced, indentations at regular intervals, which give the whole body somewhat the appearance of a rosette. Situated near the periphery is a circle of minute refractive dots, each of which occupies a position midway between two of the peripheral indentations. The indentations at the periphery become more marked and the organism divides into a variable number, roughly speaking five to twenty small bodies, which assume a circular outline. The size and shape of the segmenting bodies vary. In the larger forms within the peripheral row an internal row of segments about the pigment clump may be found.

Very frequently the corpuscle containing these large bodies shows certain changes; there is some decolorization of the stroma; the corpuscle becomes less oval, the short diameter being increased at the expense of the long; the nucleus of the corpuscle also is often apparently swollen, becoming round and less elongated. Finally, the newly formed bodies become free, and in fresh specimens of blood solid pigment clumps surrounded by groups of small spherical bodies may be seen free in the plasma. Stained by the method of Romanowsky, the segmenting bodies always take a deep color. When the process has advanced almost to division into separate segments, the body may be seen to consist of a number of approximately round, clear areas surrounded by deeply stained protoplasm; within the clear spaces may be seen blue specks which represent possibly the refractive dots of the

fresh body (Plate IX, Fig. 9). After complete segmentation there result a number of round bodies staining at the periphery and clear in the centre, identical in appearance with the youngest intracorpuscular parasites (Plate IX, Fig. 9).

As a rule the segmenting bodies correspond in size with the larger full-grown organisms, filling somewhat more than one-half of the corpuscle. Not infrequently, however, one may see in the same blood segmenting forms hardly half this size. This suggested the possibility that we may be dealing with two distinct organisms. Celli and Sanfelice think that the larger and smaller segmenting bodies belong to separate varieties; in fact, this is, as has been mentioned, the most important point of distinction between their second and third varieties of parasites, those with cycles of development of respectively mean and rapid duration. Labbé, on the other hand, because he finds the larger and smaller forms always associated, while, at the same time, others of intermediate size occur, comes to the conclusion that they are forms of one organism, a parasite which under certain circumstances may sporulate at a period somewhat earlier than usual in its life history. This explanation is in accord with what we have observed.

From analogy with what we know of the tertian and quartan parasites of human malaria it is natural that one should suspect that these organisms might, in a similar manner, undergo a cyclic development in groups, all the members of each group being in approximately the same phase of development. This, however, is apparently not the case. In a specimen of blood in which the parasites are fairly abundant one finds as a rule nearly all the forms that have been described, though not infrequently one form predominates. For this reason we have been able to arrive at no definite conclusion as to the length of the cycle of development.

In addition to the full-grown organisms, which correspond in size to the largest segmenting bodies, certain other forms of a characteristic appearance are usually to be found. These are as a rule much larger than any of the segmenting bodies and are apparently not destined to segment, at least in the ordinary way. The nucleus of the containing corpuscle is pushed to the extreme periphery; the size of the parasite

is usually such that the corpuscle is distorted. Often one finds the parasite surrounded by a rim of corpuscle from which the nucleus has been extruded. As a rule the corpuscle or what is left of it is decolorized. These bodies are much less refractive than the smaller forms and stand out, therefore, much less distinctly from the corpuscle. The pigment is not collected into a clump, but is distributed throughout the organism as discrete granules. In specimens that have been fixed and stained with eosin and methylene blue, best by the method of Romanowsky, these bodies remain almost completely unstained (Plate IX, Fig. 7); occasionally a stained peripheral rim may be made out surrounding a large unstained central area.

These are, apparently, the bodies that Grassi and Feletti have described as a separate species of organism under the name *Haemamoeba relicta*. They believed them to segment as do the other forms. Celli and Sanfelice, on the other hand, have found them only in connection with their parasites with rapid development and regard them as forms analogous to the crescents of æstivo-autumnal fever.

We have found these bodies associated with the irregular type of organism whenever the parasites were at all abundant. The fact that they are as a rule larger than any of the segmenting bodies, as well as the fact that they stain very faintly, while the segmenting bodies always contain much chromatophilic substance, is in favor of the view that they are sterile forms.

In specimens of blood containing only the irregular form of parasite we have a number of times met with flagellate bodies very closely resembling those seen in human malaria. A round extracorpuscular body, usually lying beside the free nucleus of a red blood corpuscle, is seen to acquire an active vibratory motion. This motion is due to a number of actively motile flagella which have appeared at the periphery. The flagella are similar in appearance to those which occur in the malarial parasites of human beings. They are several times the length of the diameter of the body from which they arise. At their extremities they often show slight olive-shaped swellings. Alterations in the shape of the body of the organism are common; a constriction may apparently divide it into two circular bodies touching at but

one point (Plate IX, Fig. 10), while later it again may take on its original circular shape. In time the flagella disappear and the body becomes motionless. The presence of the free nucleus beside the extracellular body indicates, in view of what is often seen to occur with the elongate parasite (p. 94), that the organism may have escaped from the corpuscle after the preparation of the specimen. The size of these extracellular parasites which develop flagella, and the fact that they are not so refractive as the smaller intracellular bodies, suggest that they are possibly identical with the large palely staining bodies with discretely distributed pigment granules.

The above described parasites we have found in the English sparrow (*Passer domesticus*), swamp sparrow (*Melospiza georgiana*) and red-winged blackbird (*Agelaius phoeniceus*). No differences were made out between the organisms found in these different species of birds.

Elongate Parasite (*Halteridium of Labbé*). The second variety of parasite which we have observed, the elongate organism which lies curved alongside the nucleus, is markedly different from the irregular form which we have just considered. Since we have found that distinct morphological differences were to be made out between the parasites of this variety present in different birds, as a type that variety will be described which was found in the greatest number of hosts. In the English sparrow (*Passer domesticus*), song sparrow (*Melospiza fasciata*) and crow (*Corvus americanus*) were found organisms morphologically indistinguishable. These may be regarded as typical examples of the halter-shaped parasite.

The youngest non-segmented organisms closely resemble corresponding forms of the irregular parasite; pigment soon appears as small brownish granules. The small forms vary in shape, usually they are round or oval. We have not observed amœboid movement, though very short processes are often present. These young forms stain only in their peripheral portions, as do the similar stages of the irregular parasite; the central part, which contains no pigment, remains unstained (Plate IX, Fig. 11). As the parasite grows it becomes elongated. Having reached a certain size it appears to adhere closely

to the nucleus of the corpuscle. When somewhat longer than the nucleus the parasite is of semilunar shape with pointed ends hugging the nucleus (Plate IX, Fig. 12). With further growth the extremities of the parasite become more bulky and rounded and finally fill almost the whole of both ends of the corpuscle. The full-grown body then lies along one side of the normally placed nucleus and curves over its two extremities (Plate IX, Figs. 13-14). The pigment of these large forms appears as very coarse oval highly refractive granules of a yellowish-brown color, distributed irregularly throughout the parasite or collected towards the two ends. The corpuscle containing the very large forms is usually somewhat decolorized, but otherwise looks normal.

Additional information as to the morphology of the organism can be obtained from specimens fixed and stained. The method which we have used most extensively is that of Romanowsky. The material which in the young forms takes up the stain increases in amount with growth, so that in the large bodies it is scattered throughout the parasite. These large parasites take a deep stain which is not, however, homogeneous, but results from the presence of a number of small oblong chromatophilic particles; these chromatophilic granules are well seen in specimens fixed by heat, stained with safranin and partially decolorized in alcohol.

In forms but little longer than the nucleus of the corpuscle there becomes visible an approximately circular, clear-cut area, which stands out conspicuously from the fact that it remains unstained. Within this pale area one or more small bodies may at times be seen which, by the methods employed, take a very faint stain. Various observers have described such a structure as a vesicular nucleus containing nucleoli.

Besides these deeply staining forms, and at times in almost equal abundance, we have found full-grown bodies which remain almost completely unstained (Plate IX, Figs. 14-16). The longest of these are somewhat larger than the deeply staining bodies. The pigment granules are situated in the two extremities; this peculiarity is explained by a further fact which staining brings out. The whole of

the middle portion of the organism is occupied by a very large, clear, almost completely unstained space, sharply marked off from an exterior layer, thickest at the two ends, which takes a faint blue stain. The sharp outline and position of the central clear space indicate that it corresponds to the so-called vesicular nucleus of the other forms. The outer faintly staining layer represents apparently the body of the parasite containing a very small amount of chromatophilic substance. It is in the latter portion that the pigment is situated, and since this portion is thickest at the extremities of the parasite, it is here that the pigment tends to collect.

When the blood is drawn large numbers of these swollen, palely staining forms quickly assume a circular outline and escape from the containing red corpuscle. In stained specimens of these circular bodies (Plate IX, Fig. 16) the central unstained area is surrounded by the external palely staining material which now forms a rim of uniform thickness. In this peripheral rim lies the pigment. In many of these bodies, which have become completely extracellular, the distinction of internal non-staining area and external palely staining material is lost and the pigment is scattered throughout the body.

The question arises whether these non-staining bodies are forms which after maturing have, by a sort of degenerative process, lost their affinity for stains, or are forms which have never acquired any considerable amount of chromatophilic substance. In favor of the latter hypothesis is the fact that smaller forms which take but little color are frequently found in the same specimen with the larger bodies.

Reproduction of the elongate form of intracorpuscular parasite does not, according to our observations, take place in the circulating blood. Neither in the English sparrow, song sparrow nor crow infected with this parasite have we found bodies showing any indication of segmentation. The bone-marrow and internal organs of these birds were not, however, examined in the fresh state.

Labbé is the only observer, so far as we know, who has definitely described the segmenting stage of the halter-shaped organism. In the lark, particularly in the bone-marrow and spleen, he found segmenting bodies. According to him the nucleus of the parasite divides into two,

the daughter nuclei occupying the two extremities of the organism. The two extremities containing the secondary nuclei then divide, each into from six to fifteen segments. We have not observed this process, although in one or two instances in the owl (*Bubo virginianus*) we have seen parasites with two of the so-called nuclei.

In the bone-marrow of a swamp sparrow (*Melospiza georgiana*) infected with elongate organisms, which to be sure differ somewhat from those which we have just described in the English sparrow, song sparrow and crows, we have, however, encountered bodies in process of segmentation. The organisms, as seen in preparations of bone-marrow teased in normal salt solution, had assumed a round form with the pigment collected into a central block, and had divided into a number of round segments arranged about the pigment mass.

From analogy with the segmenting bodies of the irregular parasite which stain well, one would suppose that it is the deeply staining bodies of the elongate parasite that segment, while for the same reason it seems improbable that the palely staining forms which are characterized by swollen vesicular body undergo segmentation.

The process of *flagellation* presented by the elongate organism is a most remarkable phenomenon. In birds in which these organisms are abundant the whole process may be followed with the greatest facility; in almost every field of the microscope (Leitz, Obj. oil im. 1/12; Oc. 3) one or more flagellate bodies may be observed. To see the whole process the specimen of blood must be prepared and brought under the microscope with considerable rapidity. Certain of the full-grown bodies which lie curved along one side of the nucleus of the corpuscle are soon seen to collect themselves into an oval, then into a circular form, causing a marked bulging protrusion from that side of the containing corpuscle. As one watches the corpuscle its body disintegrates; the hæmoglobin seems to fade away under the eye and a round organism is left lying beside the nucleus of what was shortly before the containing corpuscle. Very soon the round extracellular body acquires an active vibratory motion as the result of the presence of two, three, four, or even more, very active flagella, which suddenly make their appearance at the periphery (Plate IX, Fig. 17). The

pigment granules take on a very lively dancing motion. Changes in the shape of the body of the organism often occur. As with the flagellate forms of the irregular variety, a constriction may divide the parasite into two circular bodies connected at but one point. After a while individual flagella not infrequently become detached and float away, continuing their active serpentine movements. After persisting for some time the flagella become less active and finally disappear, by exactly what process cannot be said in every case. The pigment loses its motion.

The liberation of the intracorpuscular parasites appears to be the direct consequence of the changes which take place in the blood as a result of abnormal conditions produced in making the specimens, such as, for example, the rapid reduction of the temperature of the blood, the chemical changes in the plasma accompanying coagulation and the changes resulting from exposure of the blood to the air. It cannot be definitely determined whether the liberation of the parasite is primarily the result of disintegration of the containing corpuscle whose resistance to external changes has been weakened by its presence, or whether it may be due to the action of the parasite which, meeting with the new conditions, contracts into a globular form, and in so doing breaks from the corpuscle. It may be said that in specimens of birds' blood, however carefully made, a certain number of corpuscles which do not contain parasites disintegrate; the hæmoglobin is dissolved in the plasma and the nucleus alone remains. Where little care is used in the preparation of the specimen large numbers of these free nuclei are seen. Where organisms are contained in one of these disintegrating corpuscles they apparently become free, assuming a round form even though they have not reached the size of those which ordinarily escape. In such a case certainly the liberation of the organism is not primarily the result of its own activity, but of the distintegration of the corpuscle. A similar explanation probably holds for the larger forms.

A certain number of the organisms that become free, but by no means all, develop flagella. We have, it will be remembered, described two forms of full-grown organisms: the deeply staining bodies with small nucleus-like areas and the palely staining forms with the

large vesicular structure, which are constantly present in the blood of birds infected with the elongate type. Do both of these forms develop flagella? Since this question has suggested itself to us we have not been able to obtain a bird with sufficient number of forms capable of developing flagella to enable us to arrive at any conclusion.

If blood be allowed to stand some time before the dried preparation is made, it will be found that both the pale and the deeply staining parasites become free. This is, however, of no especial significance; under such conditions many non-parasitiferous red corpuscles also disintegrate. As far as we have been able to judge, the palely staining forms are the first to become free. Sakharoff (26), who with Danilewsky thinks that the flagellate forms are a separate species of organism, states that the organisms which flagellate are distinguished from those that do not by the possession of a very large nucleus. These bodies with large nuclei would appear to be identical with our swollen palely staining forms, which we have been rather inclined to regard as the variety which develop flagella.

Varieties of the Elongate Parasite. There is considerable diversity of opinion among observers as to the existence of distinct species of the organisms under consideration. Celli and Sanfelice observed certain special differences in the size and shape of the elongated parasites. For example, in some birds the extremities of the full-grown forms were more blunt than in others; in the barn owl (*Strix flammea*), and in the brown owl (*Syrnium aluco*), the organisms contained numerous conspicuous refractive particles scattered between the pigment granule.

Labbé, in his *Halteridia* of the lark, chaffinch, starling and jay, found differences in shape and refraction in different birds. These authors do not, however, emphasize morphological differences as indicating the existence of separate species of organisms. Celli and Sanfelice, however, think their failure to transfer organisms by inoculation from one species of bird to another, indicates that there are distinct species of parasites peculiar to the different hosts.

Labbé finds but one species of Halteridium which, in view of unsuccessful inoculation from one species of bird to another, he thinks is

composed of numerous varieties peculiar to the special hosts. Little reliance can, however, be placed upon unsuccessful inoculations from one species of bird to another as pointing to specific differences, since more recently Di Mattei, as already mentioned, has in a large number of carefully conducted experiments wholly failed to transfer infection from bird to bird of the same species.

The elongate parasites which we have encountered have presented in different birds distinct structural peculiarities which in certain cases are very striking. In several instances, however, we have observed similar differences in the organisms of a single bird. Hence, even though the morphological differences between the parasites are greater than those existing between the tertian and quartan organisms of human malaria, the lack of extended observations makes it impossible to establish distinct species. Nevertheless, we will call attention to the chief varieties which we have noted.

(1) In an English sparrow and a song sparrow obtained near Baltimore, and in three crows from near Toronto, Canada, were found organisms in every respect identical (Plate IX, Figs. 11-17). These, selected as an example of the halter-shaped organism, have already been described. They present certain distinctive features. When the young forms have reached a length but little greater than that of the nucleus of the corpuscle, unlike the other varieties, they adhere closely to the nucleus. The extremities of the organism, at first pointed, grow over those of the nucleus, hugging them closely. At this time the body is crescentic; later its two ends become rounded and bulky. The most striking feature, however, of this variety is the pigment. This is scattered through the organism as very coarse, yellowish brown, highly refractive oval granules; these in the full-grown forms are several times the size of those usually found. The large palely staining forms with swollen vesicular structure are found in great abundance. Flagellate bodies also are seen here in great numbers.

(2) In a great horned owl (*Bubo virginianus*) an organism (Plate IX, Figs. 18-22) was observed which differed markedly from that above described. In common with the other varieties it begins its growth as a small non-pigmented body, situated as a rule in one or

other end of the red corpuscle. With increase in size it often comes to occupy a large part of the extremity and may be so large as to slightly displace the nucleus, never, however, pushing it into the opposite end. At the same time growth proceeds longitudinally along one side of the nucleus toward the other end of the corpuscle. When full-grown the organism occupies every available portion of the corpuscle, displacing the nucleus laterally. The pigment consists of numerous small spherical granules which are almost black.

In fresh specimens in the half-grown and full-grown forms there may be seen between the pigment granules numerous, highly refractive, spherical particles. In specimens stained with methylene-blue and eosin these bodies appear as completely unstained round areas within the blue ground of the organism. The vesicular body described as a nucleus stands out clearly in stained specimens; within, one or more palely staining particles may be made out. Palely staining parasites are abundant. Flagellate forms with from three to five flagella are numerous. Irregularity in the shape of the flagellate body is common; bud-like processes often appear at the periphery and may give origin to flagella.

(3) In a swamp sparrow (*Melospiza georgiana*) was found an organism (Plate IX, Figs. 23 and 24) markedly unlike those already described. When full-grown it appears as a rather slender body of almost uniform width lying along the periphery of the corpuscle, at one side of the nucleus but not touching it. The border of the parasite toward the nucleus has an irregular appearance. Pigment is present in fine round granules. Conspicuous refractive bodies are not present. Although intracorpuscular organisms were seen to assume a circular shape and become free, flagellation was not observed, possibly on account of the small number of parasites present. In the bone-marrow of this bird, as already mentioned, segmenting bodies were found.

(4) In several red-wing blackbirds (*Agelaius phoeniceus*) there were seen organisms (Plate IX, Figs. 25 and 26) resembling somewhat those of the last mentioned form. They differ from these, however, in not being so slender and in their lack of uniformity in width. As

a rule they do not lie along the periphery of the corpuscle, but are often close to the nucleus. Those forms which have not completed their growth show as a rule a somewhat club-like shape, one end being more bulky than the other; growth apparently takes place along the side of the nucleus from the larger end as a starting point. In the largest forms both ends are clubbed. The pigment is in small spherical granules not infrequently collected into one or both extremities.

(5) In one red-wing blackbird (*Agelaius phoeniceus*) occurred an organism (Plate IX, Figs. 27-29) which differed from that last mentioned. The extremities of the larger forms are not clubbed, but are pointed, curling about the ends of the nucleus of the corpuscle, and with further growth approach one another on the side of the nucleus opposite to the main bulk of the parasite. In certain cases the two extremities meet one another and fuse, thus forming a complete ring surrounding the nucleus. Pigment is present as round granules, usually scattered throughout the organism, at times collected in a more or less definite clump. Associated with this variety of organism is a form (Plate IX, Fig. 29) very similar to bodies which Kruse (15) has described and pictured, regarding them as, in all probability, segmenting forms. They are seen in specimens stained by the method of Romanowsky as elongated bodies lying along one side of the nuclei; the pigment is collected into a fairly compact mass which occupies the middle portion. The body of the parasite is composed of a number of round areas which take no stain, surrounded by stained protoplasm. The whole has the appearance of being composed of a number of bodies with central, non-staining area and a peripheral staining rim. Whether or not these are really segmenting bodies cannot be definitely stated.

The above mentioned differences among the organisms observed certainly suggest the possibility of the existence of distinct species. On the other hand, it may be that a single organism is modified by peculiarities in the conditions which it meets in different species of host or in the same host under varying conditions. But even if the above described peculiarities do not indicate specific differences among the organisms concerned, they are nevertheless worthy of note as evidence of a very remarkable polymorphism.

DESCRIPTION OF PLATE IX.

The figures, those of flagellate forms excepted, are drawn from specimens stained with methylene blue and eosin by the method of Romanowsky (modified by Heppener). The figures of flagellate forms are drawn from fresh specimens of blood. Leitz, Oc. 3; Obj., oil im. $\frac{1}{12}$.

Figs. 1-10.—Irregular parasites found in the English sparrow, swamp sparrow and red-winged blackbird.

Figs. 11-17.—Elongate parasites, variety (1), found in the English sparrow, song sparrow and crow.

Figs. 18-22.—Elongate parasites, variety (2), found in the great horned owl.

Figs. 23 and 24.—Elongate parasites, variety (3), found in the swamp sparrow.

Figs. 25 and 26.—Elongate parasites, variety (4), found in the red-winged blackbird.

Figs. 27-29.—Elongate parasites, variety (5), found in the red-winged blackbird.

For fuller description of these figures see the text.

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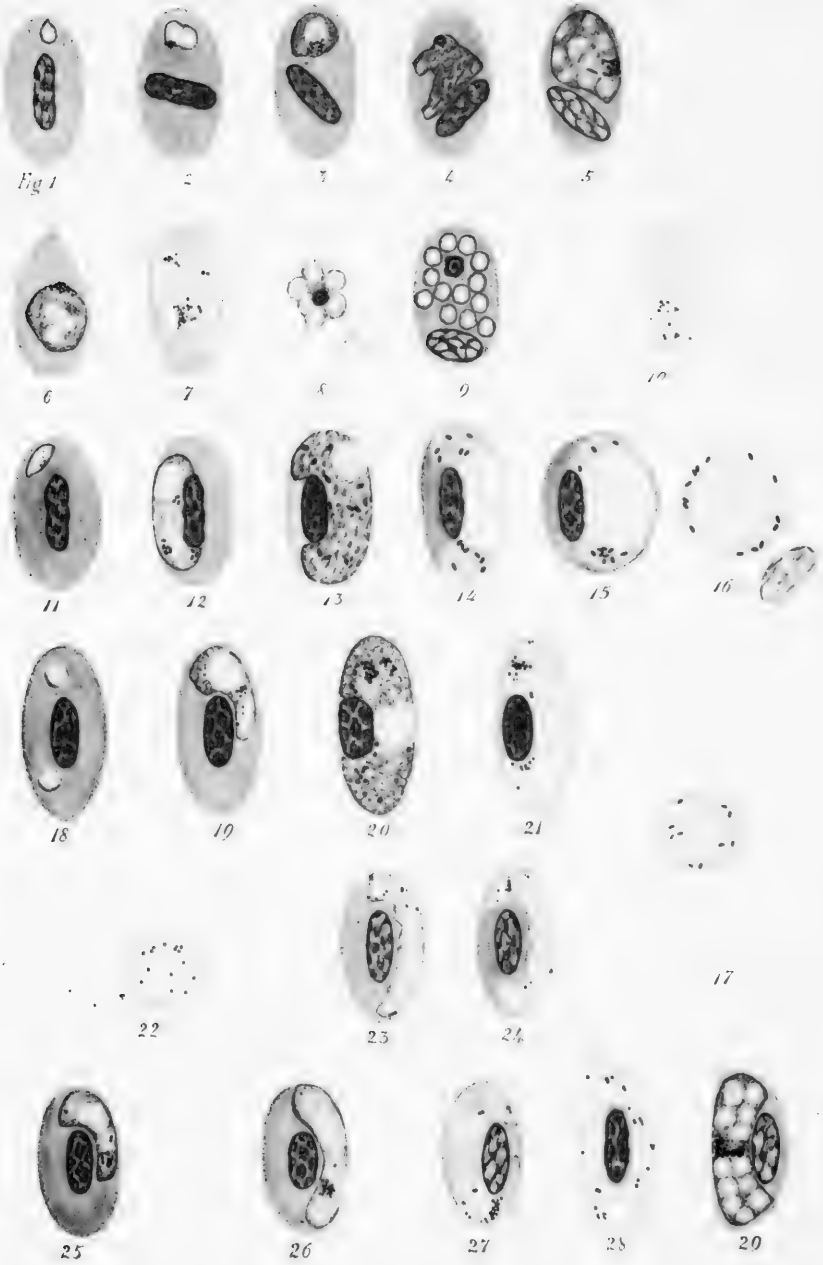
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NOTES ON THE PATHOLOGICAL CHANGES IN THE ORGANS OF BIRDS INFECTED WITH HÆMOCYTOZOA.

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PLATES X AND XI.

Several authors have given brief descriptions of deposits of pigment found by them in the tissues of birds which had for some length of time been infected with hæmocytozoa. Danilewsky, in his "Parasitologie Comparée du Sang," in 1889, speaks of the changes in the hæmopoietic organs and describes macrophages containing pigment masses, parasitiferous and shrunken red corpuscles and the remains of parasites. Again, in 1890, he describes the blackening of the spleen and the enclosure of pigmented cells in large macrophages. He found also similar changes in the bone-marrow.

Kruse (2) in the same year drew attention to the dark color of the spleen, although, owing to his lack of familiarity with the anatomy of birds, he was unable to decide whether or not the organ was enlarged. He found pigment enclosed in the large clear cells in the capillaries of the spleen, liver, lung and bone-marrow; he regarded this as an evidence of the acuteness of the process, since he argues that in a chronic infection the pigment would probably all be contained in fixed cells.

Labbé (1) in 1894 speaks also in a general way of the gross changes in the organs of infected birds.

Our own observations on the pathological changes in the organs of birds infected with hæmocytozoa are based on the results of a study of the tissues of those birds whose blood has been described by Mr. Opie in the preceding paper (p. 79). In a series of about 125 birds autopsies were made in all cases in which infection was found, and for the sake of control in a few which were free from infection. In

a number of cases, of which brief accounts are given at the close of this article, the tissues were examined microscopically.

From these descriptions it will be seen that the conditions resulting from infection with the so-called "Halteridium" and the "Proteosoma" are, roughly speaking, identical. The slight differences will be brought out in the following description of the minute appearances of the various organs.

Liver. The capillaries are not as a rule dilated. In a few instances, however (No. 31), we have observed them in places to be crowded with infected corpuscles which seemed to completely occlude the lumen. Isolated infected corpuscles are seen scattered in the capillaries throughout the liver, being more numerous in the larger vessels. Many of these are well-preserved, but according to the conditions under which the tissues were hardened, the organisms have been, in greater or less proportion, extruded from the corpuscles and lie in the capillaries as pigmented spheres, accompanied by the free nuclei of the red corpuscles. The organisms in the tissues take on a pink stain with hæmatoxylin and eosin, and present a somewhat granular appearance.

The larger masses of pigment seen in the liver tissue are contained in large cells, which vary greatly in size, shape and staining properties (Plate X, Fig. 2). They are very similar to the macrophages described as occurring in the organs from cases of malaria in human beings, and are probably analogous to these. In the liver of other animals essentially identical structures have been found acting as store-houses of pigment; the previous descriptions of their occurrence in birds in hæmocytozoan infections have already been referred to. These cells are round, ovoid or irregular structures, sometimes large enough to completely block the lumen of a capillary. That they are really in the capillaries seems to be definitely proven by some of our sections, and with this view most recent writers agree. Ponfick (12), however, in his article on the "Fate of Pigment in the Animal Body," says that in the liver of animals into whose blood cinnabar has been injected, cells similar to these and containing the cinnabar granules are to be found in the lymphatics of the vessel sheaths or, at any rate, outside the blood-vessels.

He finds also that the endothelial cells of the capillaries take no part in the storing up of pigment, a conclusion quite contrary to that arrived at by us from our observations in birds.

The protoplasm of the macrophage may remain clear and unstained; in other cases it takes on a lilac color in specimens stained in hæmatoxylin and eosin. The nucleus is always vesicular, varying in shape, being sometimes quite distorted, but oftener rounded. In some instances two nuclei have been found. Of all the varied contents of these cells the most striking is the pigment which occurs in at least two distinct forms, as follows: (1) The pigment formed by the organism—blackish-brown granules of fairly uniform size and shape, which occur separately or grouped together in spherical or irregular masses of various sizes, sometimes, apparently, far too large to be contained in a cell. (2) Straw-colored pigment, probably derived directly from the remains of the hæmoglobin which is set free into the blood stream when an infected red corpuscle breaks down and liberates the contained organism. This occurs in irregular particles, sometimes aggregated into masses similar to those just described. Although these sometimes form the only contents of the cell, many of the masses found crowding the macrophages are made up of both varieties of pigment packed together.

In other cases the large cells are filled with small masses of a fairly uniform size, sometimes irregular in outline, sometimes so closely packed together as to take on a polyhedral shape. At first sight they appear yellowish-brown, but on closer inspection are seen to be made up of a faintly colored ground substance with refractive brown granules of pigment—they are in fact shrunken parasites.

Neumann (13) and Bignami (8), in speaking of the varieties of pigment found in the organs in chronic malarial infections, recognized the fact that not only does the malarial pigment give no reaction to the ordinary tests for iron (such as Perl's reaction with ferrocyanide of potassium and hydrochloric acid or the ammonium sulphide reaction), but that, after they have been deposited in the tissues for a certain time, the iron-containing compounds derived from the hæmoglobin also cease to react. This may explain the fact that, in speci-

mens of birds' tissues which were treated with ferrocyanide of potassium, although a hazy blue stain was obtained in the cells and about the pigment, none of the fragments gave a definite blue reaction.

Treated with ammonium sulphide the pigment formed by the organisms is entirely unchanged, while in most cases some of the straw-colored variety is blackened. If, however, after the treatment with the sulphide the section be washed in water and exposed to the air, the blackened pigment becomes decolorized to a certain extent (cf. Quincke (14)), and the malarial pigment is brought out in relief.

Besides these various forms of pigment the macrophages frequently contain infected red corpuscles, which are sometimes unchanged, sometimes shrunken. In others the remains of such corpuscles form a mere debris, consisting generally of the shrunken bodies of the parasites, and finally of the broken-down remains of these with free pigment scattered in the protoplasm. The pigment is often arranged in radiating lines; sometimes it appears to lie in vacuoles. These, however, may be artefacts.

Not only the macrophages but also the endothelial cells of the capillaries may be seen to contain pigment masses (Plate X, Fig. 1). This pigment appears usually in small quantities and occurs at the poles of the nucleus. Occasionally, however, the cell is greatly distended with large pigment masses.

We are still uncertain as to the presence of pigment or parasites in any cells corresponding to Kupffer's cells in man. Many large mononuclear cells, with deeply staining nuclei and protoplasm, which takes on a lilac coloring, are found in the capillaries. Whether these can take up pigment and are to be classed with the macrophages proper we have not determined. On staining with acid fuchsin an occasional white blood corpuscle, of the class which we have for convenience termed rubinophilic cells,* may be found in a greatly swollen condition and containing pigment masses. This is, however, rather the exception, inasmuch as numerous examples of such cells are seen throughout the tissues quite free from pigment or other foreign substance.

* See page 113.

The liver cells themselves have not been observed to be phagocytic, but a very considerable pigmentation is noticeable in one or two cases (Nos. 103 and 111). Here the liver cells present a ragged vacuolated appearance and contain numerous fine straw-colored granules. On applying the ferrocyanide of potassium and hydrochloric acid or ammonium sulphide stains for iron, a sharp iron reaction is obtained and the liver presents the appearance of a close-meshed network of blue or black lines of pigment granules, according to the method used. In one liver (No. 3) there was extensive fatty degeneration.

As to the effects of the organisms or their products on the tissues but little that is definite can be stated. Necroses have been observed in the organs of several specimens, but their dependence upon the presence of the hæmocytozoa cannot be said to be proven.

We have observed two forms of focal necrosis:

(a) The first, found in the owl (No. 101), in which there was an intense septicæmia, is probably due to the presence of the bacteria. These necroses are described in the account of the autopsy (p. 115).

(b) The necroses of the second variety were found in the liver and, in a modified form, in the spleen of birds with proteosoma infection. They appear to be associated with the presence of certain oval bodies which seem to bear no close relation to the proteosoma itself (Plate X, Figs. 3 and 4). The foci in the liver are small and discrete. In the affected spots there is a complete loss of liver cells as such in the area, the centre of which is occupied by a mass of pink-staining material, which contains fragmented, deeply staining nuclei, with the debris of cells and occasional macrophages enclosing pigment and parasites (Plate XI, Fig. 1).

Among these there are often to be seen cells containing small oval bodies just about the size of the nucleus of a red corpuscle. These bodies take a general lilac color and show in their centres a ring-shaped structure which stains rather deeply with hæmatoxylin. These ovoid structures may occur free in the midst of the debris of cells and nuclei, but are more often contained in cells, where they may be seen in groups of two or more, or in larger collections of from twelve to twenty arranged with the pointed end turned to the centre and calling to mind

the appearance presented by the segmenting bodies of a malarial parasite (Plate X, Figs. 3 and 4).

These groups contain no pigment in their interstices and, from their size and general appearance, give rise to the idea that possibly they represent the segmenting stage of a protozoan parasite. This is of course only an hypothesis, and until further material is obtainable the question of their exact nature must remain undecided. Their distribution is not limited exclusively to the neighborhood of the necroses; they may be seen contained in cells elsewhere in the capillaries. They will be referred to later in discussing the spleen.

Spleen. Kruse (2) has described the changes in the spleen in infected birds. He finds that it is of a greyish-black color and contains masses of pigment within colorless cells distributed essentially in the vessels. Judging from the bright color and the great size of the granules, he believes that only one part of this pigment corresponds to that formed by the parasite. The red corpuscles taken up by the leucocytes contribute to the pigment formation, but whether the parasite first kills the corpuscle and the two are then engulfed, or whether the leucocyte swallows both alive, remains a matter of doubt.

Owing to certain peculiarities in the distribution of the pigment in the spleen it is desirable to consider certain details in the normal anatomy of the spleen of birds before entering upon a description of the pathological changes. Wilh. Müller (6) and A. J. Whiting (7) have given the most satisfactory accounts of the structure of the spleen of birds; their observations differ in no essential points from those made by us. The capsule, at least in the smaller birds, possesses no trabecular system; the vessels, however, are surrounded by a certain amount of connective tissue. In the neighborhood of these masses of connective tissue the pigment is especially deposited. At the points of branching of many arterioles spherules of lymphoid tissue are seen placed laterally, sharply marked off from the adjoining tissue by a double row of elongated cells, which Whiting considers to be muscular, but which, since they sometimes take up pigment, seem to us to resemble rather endothelial cells. These do not occur in sparrows.

The further prolongations of the arterioles show here and there

changes in the nature of their endothelium, the nuclei of which become large and vesicular. In these places the adventitial layer becomes greatly thickened so as to form an ellipsoidal mass of pink-staining tissue, in which are embedded large vesicular nuclei. Associated with these structures there is a considerable amount of lymphoid tissue poor in capillaries, the round cells of which merge gradually into the large cells of the pulp proper, which runs in bands among the spherules and ellipsoids. These pulp bands are made up of wide capillary spaces which anastomose freely in a network of large pulp cells, characterized by large vesicular nuclei of round, oval or polyhedral form, and with slightly opaque protoplasm which takes a pink stain. These cells are most closely aggregated at the margins of the bands of pulp, where in hæmocytozoan infections they become conspicuous from their actively phagocytic properties. Sometimes, as in case No. 101, they become so loaded with pigment that the pulp bands appear as a black network on section of the spleen (Plate XI, Fig. 2). They exist in numbers directly under the capsule, where again they may contain a conspicuous collection of pigment. The extent of the pigmentation varies greatly, as will be seen from the autopsy reports. The nature of the deposit, however, and its distribution is similar in all, the pigment resembling exactly that described as occurring in the liver, and giving the same staining reactions.

The proportion of the varieties of pigment differs to a certain extent in the different specimens; for example, in No. 104 the pigment is mostly yellow, while in Nos. 5 and 103 it is mainly of the dark variety; this is, however, possibly dependent on the duration of the process, the yellow pigment being, as Bignami has suggested, changed by a local reaction into a dark material which does not respond to ordinary tests for iron. Almost all of this pigment is contained in the large cells mentioned above as forming the groundwork of the vascular bands, and most closely arranged along the margins of these pulp bands, directly under the capsule and in the neighborhood of the large vessels. As a direct result of this arrangement we have a network of pigmented cells, as shown in Plate XI, Fig. 2.

As to the exact nature of these cells we cannot be quite certain; but since our observations agree with those of Müller, who finds no defi-

nite endothelial lining to the capillary blood spaces, it seems probable that these cells, which themselves form the limits of the blood spaces, are to be considered as essentially endothelial in nature. Their form, when they have acted as phagocytes, is very similar to that of the macrophages seen in the capillaries and larger vessels of the spleen, liver and other organs. Distended sometimes to a great size and crowded with pigment balls, pigment granules, infected red corpuscles and debris of various sorts, their nuclei swell and become pale, while the protoplasm often becomes clear. Deposits of pigment also occur in the elongated cells which outline the spherules, as a result of which they sometimes become greatly distended.

Degenerative changes have also been observed in the spleen. Those occurring in connection with the bacterial infection in case No. 101 are identical in character with the necroses observed in the liver of that case.

In two cases (Nos. 103 and 111) a considerable fragmentation of nuclei was noted, more especially in the lymphoid spherules, where the bulk of the nuclei are represented by very deeply staining dots. The cells have lost their contour and the whole spherule has a degenerated appearance.

Quite a different picture is presented in the spleens of Nos. 5, 80, 31, and some others where there are well-defined annular hyaline deposits which stain bright pink. These rings often enclose groups of large macrophages containing yellow pigment. In the cases of proteosoma infection there are found also in the spleen the peculiar ovoid bodies referred to above in the description of the liver, although none of the above lesions can be definitely ascribed to their presence. These present the same appearance as in the liver, excepting that they are more frequently arranged in beautiful symmetrical groups as in a segmenting organism. These groups vary much in size, the segments numbering from 5 to 20 or more; as in the liver, they occur within large cells, which here may be definitely identified as cells of the splenic pulp. They are most numerous in the neighborhood of the ellipsoids and generally in the less richly vascular portions of the spleen. In case No. 43 there are areas in which almost every cell contains a group, some cells containing even two. It is not improbable that some light

upon the nature of these bodies may be obtained later by aspirating the spleen of infected birds during life.

Bizzozero (5) has given a very good account of the structure of the *bone-marrow* in birds. It is unnecessary, however, at the present time to review his work. All writers upon the subject who have so far referred to the pigmentation of the organs in birds, have especially noted the dark color of the bone-marrow. Danilewsky (3) describes large protoplasmic bodies containing pigment and fragmented red corpuscles, and again (9) numerous "macro-melanophages." Labbé (1) and Kruse (2) find pigment in the capillaries of the bone-marrow in virulent cases.

Our own experience has not impressed us with the intensity of the pigmentation in the bone-marrow. Unfortunately, in case No. 101, where there was a most intense infection and where the other organs were deeply pigmented, the bone-marrow was lost; but in the succeeding cases where it was carefully studied no accumulation of pigment was observed, and but few organisms as such were found in the capillaries. In case No. 3 numerous organisms were seen, but no pigment blocks, and it was only in Nos. 81 and 31, where smears were made from the bone-marrow, that large pigmented cells were found in moderate numbers. These with their contents were in most respects similar to the large phagocytic cells seen elsewhere, with the exception that the pigment was in smaller masses.

But little remains to be said of the *visceral changes*. The brain in all our cases was quite normal; of course intra- and extra-corpuscular organisms were occasionally seen in the capillaries, but they were never collected in masses sufficient to occlude the lumen of the vessel. There was no pigmentation of the tissues except that a few doubtful pigment granules were situated at the poles of the nuclei of some of the endothelial cells in the capillaries in case No. 31.

Organisms were found in the capillaries of the lungs, but beyond inspired carbon particles no further pigmentation of the lung was to be made out. This is contrary to the experience of Kruse (2), who always found pigmentation of the lungs. This coal pigment is of a dead black color, readily distinguishable from either of the forms of pigment described as the result of the infection. Chemically it is not

acted upon by any of the reagents employed to demonstrate the presence of iron. It is distinguishable in the neighborhood of the bronchi and air sacs and is evidently in close relation to the lymphatics.

The stomach (proventriculus) and intestine were quite normal in all the cases with the exception of the presence of a few organisms in the vessels of the mucosa.

The kidneys showed occasionally a few intravascular organisms, but were otherwise normal.

The pancreas, thyroid, heart and muscles showed nothing abnormal.

Phagocytosis, which apparently bears a close relation to all this pigmentation of the organs, has not been very satisfactorily studied in the blood. Labbé (1) denies the existence of an active protective phagocytosis; wherever he found infected corpuscles within the leucocyte the normal elements were associated with them. He admits, however, that parasites are engulfed and destroyed by the phagocytes and more especially by those of the hæmopoietic organs.

Kruse (2) has observed the engulfing by the leucocytes of the pigment left by the degenerated flagellated forms.

Danilewsky (3) followed carefully the changes seen in the infected red corpuscles on being engulfed by an active phagocyte. He finds that the stroma of the red element is first slowly digested away, leaving the intact organism, and that the organism then loses its sharpness of contour and is finally lost to view, leaving only the pigment.

The morphology of the leucocytes as given by Bizzozero (4) is briefly as follows:

(1) Cells 6.5 to 7.8μ in diameter with rather large polymorphous nucleus and clear protoplasm, in which are contained numerous highly refractive fusiform rodlets which take on an eosin stain.

(2) Cells of the same size with similar nucleus and protoplasm, but with small spherical eosinophilic granules—cells almost like human eosinophiles.

(3) Small leucocytes with large spherical nucleus and homogeneous protoplasm.

(4) Large leucocytes 6.7 to 8.3μ in diameter, spherical or oval, with large nucleus and opaque finely granular protoplasm.

Of these we have found that the first are the more common, corres-

ponding apparently in numbers to the polymorphonuclear neutrophiles in human blood. For the sake of brevity in description it has seemed to us advisable to adopt some special term to distinguish this variety of cell. The greater richness with which these particular granules stain with acid fuchsin has suggested to us that the name *rubinophile* might not be unfitting, and it is by this term that we shall hereafter refer to them. They are evidently the chief agents concerned in phagocytosis in the circulating blood. After watching for a long time, we have observed that they sometimes engulf degenerated organisms and present then the same appearance as the pigmented leucocytes in human blood. In the fresh slide we have several times observed the leucocyte, after partly surrounding the organism, to leave it in a still further disintegrated condition and wander away. It appeared to engulf the protoplasmic remains and leave the pigment free in the plasma. Even in such an intense infection as that which had occurred in the owl (No. 101), where several organisms could be seen in every field, we found no pigmented leucocytes in the fresh blood; nor have we met with pigmented rubinophilic leucocytes in the vessels of the tissues except in one or two instances, although in clots of blood taken from the aorta we have occasionally found macrophages loaded with pigment and the detritus of organisms. The exceptions referred to were demonstrated in the liver of case No. 101 by staining with acid fuchsin. In these specimens two rubinophilic cells very much swollen and containing masses of pigment were found in the capillaries.

In these changes there is seen a marked general resemblance to those occurring in the organs of human beings suffering from malarial infection, changes which have been so accurately described by Barker (10). The chief point of difference is in the behavior of the white blood corpuscles, which appear here to play but little part in the changes in the tissues. In no instance have we observed the inclusion of the leucocyte within a macrophage and, although in the liver of case No. 81 there were great accumulations of rubinophilic cells, not one is seen to contain pigment or the remains of an engulfed parasite. The intimate nature of the different pigments here found and their

relation to that found in the tissues of human beings with malarial infection merits careful chemical study.

In conclusion I wish to express my thanks to Dr. Thayer, to whom I am indebted for many kindnesses and much substantial assistance in this work, which was undertaken at his suggestion and continued under his supervision; also to Dr. Barker, who has examined my preparations and given me many valuable suggestions.

The main points in the results of the autopsies may be briefly summarized as follows:

(No. 3.) *Passer domesticus* (English sparrow); Halteridium infection. *Liver* mottled, dark reddish brown; shows fatty degeneration and slight pigmentation.

Spleen greyish white; microscopically, shows few organisms and very little pigmentation.

(No. 5.) *Passer domesticus;* Halteridium infection. *Spleen* enlarged; bluish black. Microscopically, shows bands of black pigment corresponding with the pulp bands and an extensive subcapsular deposit. The dense pigment masses are intracellular. *Liver* somewhat pigmented; the large cells containing pigment lie in the capillaries. The other organs normal in appearance.

(No. 31.) *Passer domesticus;* Proteosoma infection. *Liver* large, homogeneous, dark brown. Microscopically, the capillaries of the liver are often found crowded with infected corpuscles. There are areas of focal necrosis with central hyaline deposit, loss of liver cells and many distorted nuclei (Plate XI, Fig. 1). In the capillaries surrounding these, but much oftener in the large intracapillary cells, are found ovoid bodies which have been described above. *Spleen* enlarged, black; much pigment on teasing; densely pigmented in a characteristic way and presenting annular areas of hyaline change. Numerous groups of the ovoid bodies may be found here also.

(No. 43.) *Passer domesticus;* Proteosoma infection. *Liver* large. *Spleen* 20 x 3 mm.; black. There is extensive pigmentation of both liver and spleen, and great numbers of the above-mentioned ovoid bodies are found in both organs (Plate X, Figs. 3 and 4). Post-mortem changes have masked the effects on the tissues.

(No. 80.) *Agelaius phoeniceus* (blackbird). *Liver* dark in color; slightly pigmented. *Spleen* enlarged, black. There is moderate pigmentation and very numerous regular symmetrical groups of ovoid bodies are to be seen.

(No. 81.) *Passer domesticus;* Halteridium infection. *Liver* light brownish red. Microscopically shows areas crowded with leucocytes and round granular eosinophiles; pigmentation slight. *Spleen* greyish brown; not enlarged; some pigment seen on teasing. Much pigment in bone-marrow.

(No. 101.) *Bubo virginianus* (horned owl); Halteridium infection. Bird lived some time with a broken wing and with shot in its lungs and other organs. *Liver* chocolate brown; microscopically deeply pigmented; capillaries contain numerous macrophages and other phagocytic cells. *Spleen,* 1.5 x .7 cm. (twice normal size); microscopically shows most intense pigmentation corresponding to the margins of the pulp bands, so that on section it presents a coarse network of black lines (Plate XI, Fig. 2). Bone-marrow unfortunately not preserved. In all the organs there are focal necroses in which are seen masses of bacilli of a rod-like form, staining weakly and occurring sometimes in threads. They are also found distributed in the capillaries and unassociated with necroses. Cultures were not made. This septicæmia was probably a terminal infection.

(No. 103.) *Corvus americanus* (crow); Halteridium infection. *Spleen* dark red; microscopically shows areas of cellular degeneration with fragmentation of the nuclei. These are limited to the spherules of lymphoid tissue. *Liver* normal in size and color; pigmentation not extensive; a few rubinophilic leucocytes are seen to contain pigment in the liver capillaries.

(No. 104.) *Passer domesticus;* Proteosoma infection. *Liver* brownish; microscopically, definite focal necrosis associated with the ovoid bodies, as in case No. 31. The pigment is in small dense masses and moderate in amount. *Spleen* large; 10 x 2 mm.; bluish red in color; ovoid bodies similar to those in the liver are to be found also in the spleen.

(No. 111.) *Corvus americanus;* Halteridium infection. Organs looked fairly normal. *Spleen* rather dark in color; microscopically, pigment is not abundant and has the usual distribution; occasional areas of degeneration in the lymphoid nodules. The liver cells, as in No. 103, are vacuolated and contain much fine yellowish pigment, which gives the iron reaction. Bone-marrow dark reddish brown; no pigment masses and very few organisms are to be seen.

(No. 119.) *Corvus americanus;* Halteridium infection. *Spleen* blackened; organs otherwise of normal appearance. *Liver* shows slight pigmentation; few macrophages and endothelial cells containing masses of pigment. Bone-marrow negative. There was here, as in No. 111, a coincident infection with filaria.

DESCRIPTION OF PLATES X AND XI.

PLATE X.

Fig. 1.—Portion of the liver of an infected bird showing a pigmented endothelial cell.

Fig. 2.—Macrophages loaded with pigment, etc., as seen in the spleen and liver.

Fig. 3.—From the liver of case 43, showing the intracellular ovoid bodies described p. 107.

Fig. 4.—An isolated liver cell containing a group of the ovoid bodies.

PLATE XI.

Fig. 1.—From the liver of case 31, showing a focal necrosis with loss of liver cells, which are replaced by hyaline material. In many places groups of the ovoid bodies can be seen in the necrotic tissue.

Fig. 2.—From the spleen of case 101, showing the distribution of the pigment. Focal necroses caused by bacteria are also seen in the pulp.

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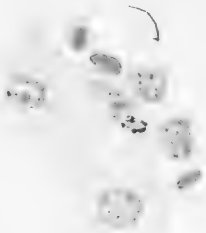

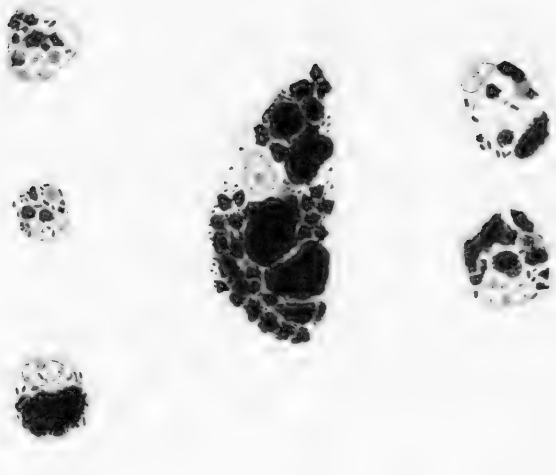

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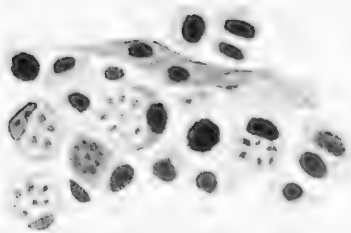

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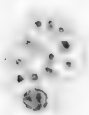

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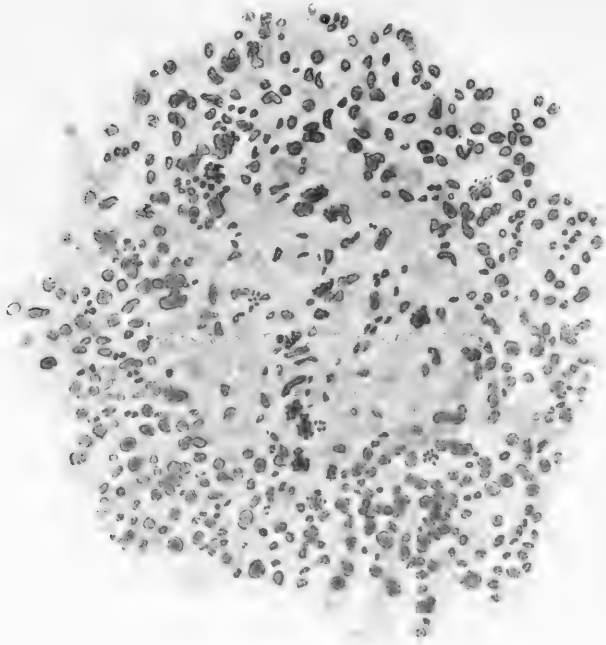


Fig. 1

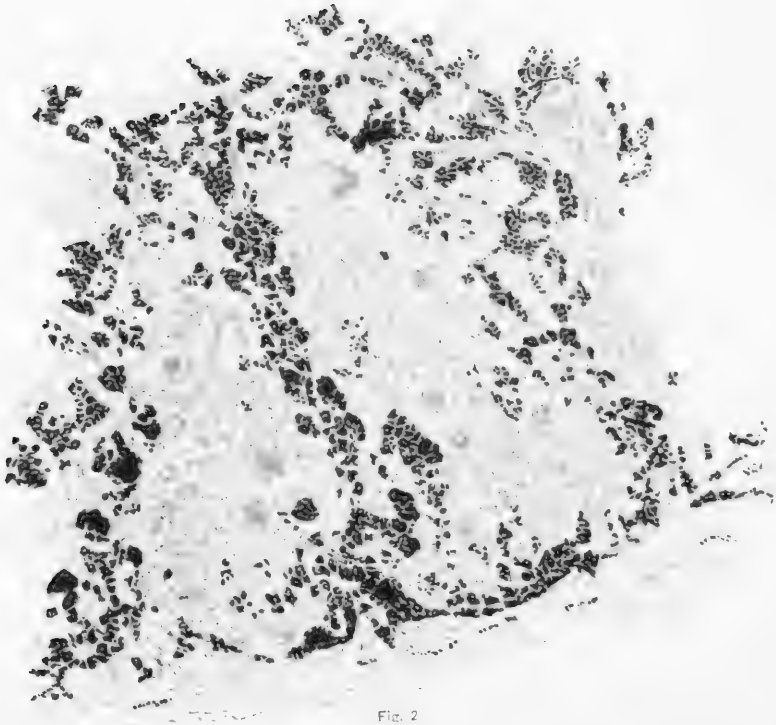


Fig. 2

ON THE HÆMATOZOAN INFECTIONS OF BIRDS.

By W. G. MacCALLUM, M. D. (Johns Hopkins University).

Plate XII.

Our knowledge of the nature of these infections, sometimes loosely designated as avian malaria, is mainly due to the researches of Danilewsky, Laveran, Kruse, Sakharoff, Grassi and Feletti, Celli and Sanfelice, Pfeiffer, Di Mattei, and Labbé. The only work upon this subject so far done in America was reported in the papers on the etiology and pathology of hæmatozoan infections in American birds, read by Opie and myself before the Medical Society of the Johns Hopkins Hospital in November, 1896, and printed in brief in the *Bulletin of the Johns Hopkins Hospital* for March, 1897. These papers, which are published in full in the present number of this Journal (pp. 79 and 103), give the results of observations made at the suggestion of Dr. Thayer of the Johns Hopkins Hospital, in the summer of 1896, on the blood and organs of various birds.

During the past summer (1897) I have continued the work in Dunnville, Ontario, confining my attention mainly to crows, which seem to afford the most promising field for observation.

Many species of birds have been found to be infected with these organisms to a greater or less degree—in Europe sparrows, finches, starlings, blackbirds, crows, hawks, etc. In Ontario and Maryland, where we have procured our birds, we have found sparrows, blackbirds, owls and crows infected, and of these the crows were most frequently so, for although the number is too small to furnish any definite conclusions as to relative frequency of infection, some idea of this may be gained from the fact that of eighty sparrows the blood of five showed organisms, while of twenty-one crows sixteen were infected.

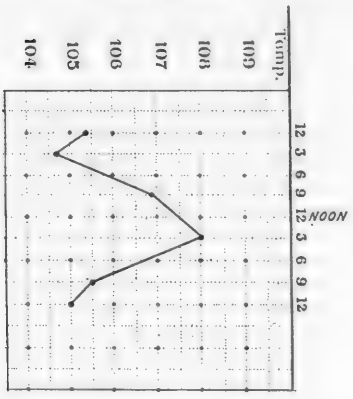
The organisms in these cases are very similar in many respects to those found in the blood of human beings suffering from malaria.

There are, however, certain well-marked differences which I shall briefly review before detailing the results of my work of the past summer. According to the observations of Labbé,* and those of Opie,† two types of organisms may be distinguished differing in form and life history as well as in the effects produced on the host. Of these the first, named by Labbé "Halteridium," begins its cycle of existence as a small oval or rounded hyaline body, situated laterally or at one end of the nucleus of the red corpuscle. As it increases in size it extends in a direction conditioned by the position of the nucleus, at the same time by its metabolic activity forming in itself granules of blackish-brown pigment. As it grows it extends more and more along the sides of the elliptical nucleus until finally, on reaching its adult stage, it is seen to coil about the nucleus, embracing it quite closely and expanding at each end into a wider portion into which the pigment, which increases with the age of the organism, is often gathered. Stained specimens show a tingeable cytoplasm in which the pigment is embedded and within which lies a clear non-stainable body with a central stained spot. This clear body is thought to be the nucleus with central nucleolus, and plays an important part in the process of segmentation as described by Labbé. He finds that the nucleus of the parasite divides into two segments, which separate and proceed to the ends of the organism, where they divide into multiples of two, thus forming two rosettes of segments lying at the ends of the red corpuscle. In the middle of the red corpuscles beside the nucleus there remains the pigment together with some debris of the organism. Opie has not been able to confirm this observation, but has seen the formation of single rosettes in the bone-marrow. I have also found forms which might be designated as pre-segmenting in the circulating blood. In these the organism had rounded itself off in one end of the corpuscle, the pigment being gathered into a mass in its centre, the margin showing a number of indentations corresponding with which there were the fine refractive dots thought to represent the nuclear flecks in each segment.

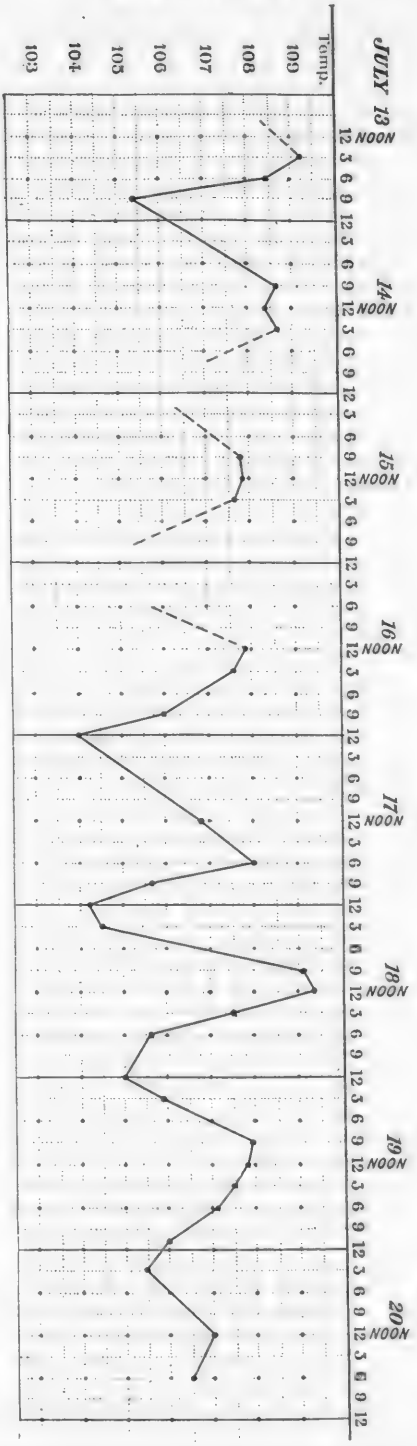
* *Arch. de zool. expér.*, série 2, 1894.

† The present number of this Journal, p. 79.

Chart I, showing Daily Temperature of a Normal Crow.



Temperature Chart II of Crow infected with Halteridium.*



Flagellation almost identical with that observed in the case of the human malarial organisms is very readily seen in these organisms. The exact length of the cycle is very difficult to determine, because all the organisms present in the blood of any bird do not segment at the same time, nor even, so far as can be determined, in large groups, but at any time almost all stages can be seen. Labbé places the cycle at seven to eight days. He, as well as Danilewsky,† states that very little can be observed clinically in infections with this form of organism, although in the case of the second form about to be described, there are febrile symptoms with a certain rise in temperature. I have attempted to construct the accompanying chart from a series of observations on the temperature of a crow infected with the Halteridium and, although it does not extend over any great length of time, it will serve to give some idea of the course of the temperature as compared with that of a crow which showed no infection. There is seen to be a daily rise of from 3 to 5

* Kindly charted for me by Miss Hutchinson of the Training School for Nurses of the Johns Hopkins Hospital.

† *Arch. f. Hyg.*, xxv (1895), 227.

degrees from the temperature recorded at midnight, and this, to a certain extent, is true of the normal crow, so that it can hardly be ascribed to the effect of the organisms, but we must perhaps rather consider the crow to be, to a certain extent, poikilothermic.

There is, however, a rather higher temperature recorded on July 13, 18, etc., suggesting that some group of considerable size might be segmented at intervals of five days. This, however, is not very constant and may have been accidental, although the blood on these days of high temperature seemed to show a larger percentage of hyaline forms.

A variety of this Halteridium is found in the crow, blackbird, etc., in which the organism is much more slender, and when full-grown finds room to lie in the corpuscle without touching the nucleus.

The second type of organism, named by Labbé "Proteosoma," also begins its cycle as a small, rounded or ovoid, hyaline body, generally situated near one end of the red corpuscle. As it grows, instead of accommodating itself to the position of the nucleus, it causes such a change in the stroma of the corpuscle that the nucleus no longer retains its central position, but is pushed to the opposite end of the corpuscle, where it comes to lie transversely. The pigment, which is on the whole finer and blacker than that of the preceding form, makes its appearance early and is distributed over the body of the parasite, which, in its adult stages, takes on a rounded or irregular polygonal form. Flagellation occurs precisely as in Halteridium. Segmentation takes place in the circulating blood and results in the formation of a simple rosette with a central mass of pigment. As stated above, this form of infection seems to be accompanied by more serious clinical phenomena than the other.

The Proteosoma I had no opportunity of observing during the past summer, and it is more especially of the Halteridium, which occurs so abundantly in crows, that I here wish to speak. The infections with the latter organism are generally relatively slight—one or two organisms being found in a slide of blood—and the birds may show no external evidence of being sick. There are other cases, however, where the bird can be recognized at a distance as being sick by its evident weakness, its uncertain flight and altered voice, as well as its draggled ap-

pearance and lustreless plumage. Such birds often show an enormous infection, at times more than a third of the corpuscles being invaded, with sometimes two or even three parasites in a single corpuscle. They may, however, recover to a certain extent in confinement when well fed, and the organisms may be seen to diminish in number most markedly.

On examining a slide of fresh blood where the organisms are numerous, examples of flagellating forms are observed with extreme readiness about two and a half minutes after the blood has been taken from the bird (Plate XII, Fig. 6). This process, accurately described by most authors on the subject, takes place as follows: The adult organism is seen to draw itself together into a sphere within the red corpuscle, and sometimes immediately, but more often after a short delay, it begins to be greatly agitated, the pigment dancing about and the surface of the sphere taking on an active undulating motion which lasts but a short time, for the organism suddenly bursts from the corpuscle, scattering the remains of the latter, and in its place beside the nucleus of the corpuscle, which now lies free in the plasma, it throws out four or more flagella, which thrash about wildly and sooner or later become detached and wriggle away. The sphere is much reduced in size by this throwing out of flagella and the pigment is concentrated, although occasionally a pigment grain is included in one of the flagella, which are not perfectly regular in form, but may branch or run into one another at the base. Their number is not constant and may change in the process of their formation. The remains of the sphere continue to be agitated and, after the loss of the flagella, its pigment sets up a most active dancing. Often it constricts itself into two or more parts, which may reunite, and sometimes a more than usually stout prolongation of protoplasm, which has failed to become a perfect flagellum, may adhere and beat about for a long time. Disintegration and death are the inevitable fate of these remains of the flagellate body, even if it escapes for any length of time one of the voracious leucocytes which wander about.

There is a certain variation in the relation of the act of throwing out flagella to the extrusion from the corpuscle. At times the organ-

ism can be seen to throw out flagella while still within the corpuscle, these beating the nucleus about within the enveloping membrane of the corpuscle, which is violently agitated and distorted like a loose bag containing the struggling organism. At last the membrane bursting liberates the fully developed flagellate form. In other cases the sphere slowly escapes through a rent in the capsule, and after a period of quiescence outside the corpuscle, begins to be agitated and finally to put forth its flagella.

Other organisms escaping quietly in this way through a rent in the capsule remain quiescent and never become flagellated (Plate XII, Fig. 5).

There is always in these processes the escape of the remaining hæmoglobin of the corpuscle into the plasma, with a corresponding blanching of the corpuscle and, although the thin capsule is left for a time, it soon becomes invisible.

Many theories as to the nature of these flagellated forms and the significance of the process have been put forward, none of which have met with general credence. Laveran thought the flagella were pre-existent in the body of the intracorpuscular parasite. He believed the flagellate forms of the human malarial organism to be the final and most perfect stage of the parasite, and that they developed within the round and crescentic bodies which he regarded as cysts. Dock considered that they represented a resting stage of the organism capable of existing independently, perhaps even of reproducing themselves, but also capable under favorable circumstances of reproducing a typical growth of the parasite. Mannaberg suggests that they are organs which permit the parasite to enter into a saprophytic existence. "I suspect," he says, "that the flagellate bodies enter upon the first stages of a cycle of existence outside of the human body, and that as a result of the unfit culture medium the death of the young spores occurs." The whole Italian school believes them to be due to degenerative changes. Manson, as is well known, has advanced the idea that the flagellate bodies represent the forms in which the parasite exists outside the human body, that the flagella penetrate from the stomach into the body of mosquitos which have sucked the blood of

infected human beings, and that, after a further unknown process of development, they come again, through the water in which the mosquitos deposit their eggs and die, into the human body. Sakharoff recognizes the flagellate bodies as dying forms; he finds the large red nucleus (with Romanowsky's stain) with a clustered chromatic filament. In the free forms this last breaks up into several filaments, which leave the parasite as flagella—a perversion of karyokinesis which is provoked by physico-chemical means. Zacharias claims to have produced flagella in epithelial cells by the action of phosphoric acid, and Labbé gives it as his opinion that the process of flagellation resembles a chemical one, that lack of oxygen hastens the process, as also does the sudden drop in temperature from 42 to 15 degrees C. Flagellate forms, he concludes, are abnormal agony products preceding degeneration, which do not exist in the living blood, but form outside the body under the diverse physico-chemical influences which modify the plasma and corpuscles obtained from the vessels.

It will be seen that none of these ideas, except that of Sakharoff, are based on any observations, but are for the most part pure hypotheses. That the flagellate forms are the agony products of the dying parasites seems to be the most generally accepted idea, and this, it is true, has the fact to support it that it occurs only under unfavorable circumstances, and is followed by the death of the organisms.

Other motile bodies have from time to time been described as occurring in the blood of birds. Danilewsky* describes elongated non-pigmented organisms found in fresh blood plasma. These are seen to swell and contract and frequently to advance in a curved or spiral line. He thinks they are related to the Drepanidia, but Labbé suggests with more probability that they are free flagella.

In addition to these Danilewsky also describes an organism resulting from the transformation of an intra-corpuscular pigmented form. This becomes extra-corpuscular and spherical and, after the lapse of ten to thirty minutes, a process appears at the periphery of the body which, increasing in size, gives rise to a curved elongated body which he calls a vermiculus. This body, which has acquired a nucleus,

* Danilewsky, Parasitologie comparée du sang. Kharkow, 1889.

moves about in a curved line in the plasma. Kruse * described a similar form in the blood of a crow, and Pfeiffer † in that of an owl. No further idea as to their nature was given.

It was noted by Opie (p. 92) that there were variations in the adult form of the Halteridium, which I have endeavored to depict in Plate XII, Figs. 1 and 2 and Figs. 3 and 4. This difference, which sharply distinguished two forms of the organism, consisted in a granular appearance in the one as compared with the clear hyaline protoplasm of the other forms. The protoplasm in the first form was not simply finely granular, but in a groundwork of finely granular protoplasm could be seen minute hyaline points. This difference was much more sharply brought out by staining with methylene blue when the granular form took on a deep stain, the fine granules especially appearing dark blue while the hyaline points remain wholly unstained. The hyaline form, on the other hand, took almost no stain, the slight tinge of blue being for the most part peripheral, sometimes with a blue fleck in the centre of the unstained portion. The pigment granules are, as a rule, finer in the granular forms than in the clear hyaline forms.

Opie (p. 96) put forward the suggestion that it might be these clear non-staining forms which become flagellated, the granular forms being the ones observed to degenerate without flagellation.

My observations have enabled me to confirm this distinction, and to demonstrate that it is true that the granular forms never become flagellated and that the clear forms alone do so. Indeed, it may be prophesied of any organism while yet intra-corpuscular that it will or will not become flagellated.

While watching one of these specimens which had stood for some time I was surprised to see an elongated organism moving slowly across the field followed by others moving in all directions. These were evidently modifications of the organism familiar in fresh specimens, and the question of greatest interest was to show their relation to the other forms. They corresponded very closely with the vermi-

* Kruse, Virchow's *Archiv*, cxx (1890), 541, and cxxi, 359.
† Pfeiffer, *Zeitschr. f. Hyg.*, viii (1890), 309.

culi of Danilewsky, and I have assured myself since that they are identical with them.

As the forms which flagellated were always observed to disintegrate or succumb to a leucocyte, the idea remained that the motile forms might arise from the forms described as being extruded from the corpuscle without flagellation. On carefully watching these for fifteen to twenty minutes it proved to be easy to see the change from the non-motile sphere into the motile elongated organism or vermiculus. In one or two instances, however, it was noted that such non-motile spheres apparently became flagellated without the extreme agitation usually seen in the process of flagellation, and without any diminution in their bulk, and later on gave rise to the motile forms. This seemed to contradict the idea that these arose exclusively from the non-flagellating forms, and apparently there remained the conclusion that flagellation was a process which proceeded more or less violently in different cases; that where the violence was great the store of energy of the organism was exhausted; where, however, it did not take place, or took place in such a modified and mild form that not much energy was expended, the organism was able to take on a motile form.

I decided, however, on the impulse of another idea, to observe carefully in the same field a granular form and a hyaline form from the time of extrusion from the corpuscle to the beginning of the motile stage, and, having found such a field, the following picture presented itself: The two forms lay at some distance from one another separated by the plasma and a few corpuscles. The granular form happened to escape from the corpuscle first and lay perfectly quiet beside the free nucleus and the shadow of the corpuscle. Soon the hyaline body, becoming greatly agitated, burst from the corpuscle and threw out active flagella, which beat about for a few moments and finally tore themselves loose. Then came the acme of the process. One of the four flagella passed out of the field, but the remaining three proceeded directly toward the granular form, lying quietly across the field, and surrounded it, wriggling about actively. *One of the flagella, concentrating its protoplasm at one end, dashed into the granular sphere,*

which seemed to put out a process to meet it, and buried its head, finally wriggling its whole body into the organism, which again became perfectly round. The remaining flagella, seeking to repeat this process, were evidently repulsed, and soon became inactive and degenerated. Immediately on the entrance of the flagellum the pigment of the organism was violently agitated, without, however, any disturbance of the outline of the organism. Soon all became quiet again and the period of quiescence lasted for about fifteen minutes, when a conical process began to appear at one margin of the organism, which, increasing in size, drew into itself most of the protoplasm, the pigment to a certain extent being gathered into the remainder. Finally most of the pigment was concentrated into a small round appendage, which remained attached to the end of what now had became an elongated fusiform body, which soon swam away with a gliding motion.

Have we not here, without much doubt, a sexual process in the organisms the result of which is the motile vermiculus? This is a process which we might have expected and which I am confident will be found to occur in the case of the human malarial parasites, although on account of the small number of organisms to be found in each slide in the latter case, the completion of the process will rarely be observed. It is entirely analogous to the sexual process as it occurs in the lower plants under unfavorable conditions. In Spirogyra and other related algæ, when their circumstances have become such that the ordinary reproduction by fission can no longer result in the successful prolongation of life, a more resistant body is formed by the conjugation of the adjacent cells of two filaments; but so long as conditions of growth are favorable we look in vain for this process. A similar conjugation is observed in many of the Ascomycete fungi and many other plants. In the animal kingdom we find it again and again, and I need only mention the pond Amœba as a familiar example.*

* Since the communication of this paper to the British Association for the Advancement of Science in Toronto on August 24, 1897, I have been much interested in the perusal of the article by Simond (*Annales de l'Institut Pasteur*, July 25, 1897, p. 545), in which he describes in certain coccidia a process analogous to that described here and suggests the probability of the occurrence of a sexual process in the malarial organism.

We can thus consider the two forms of adult organism found in the fresh blood as male and female, the granular female form containing more chromatophilic substance than the hyaline male form, which we may now say gives up its life in the production of four or more spermatozoa or flagella. Now, too, the mistake of thinking that the motile forms might arise from organisms in which the process of flagellation had proceeded less violently, is easily explained. Such organisms were simply the spherical granular forms surrounded by spermatozoa or flagella, which had arrived from the neighboring true flagellate forms and were undergoing the process of fertilization.

In view of all this it seems to me that we must look on the observations of Sakharoff with more confidence than has been accorded them by authors in general. Although I have stained with an excess of gentian violet the free flagella in the plasma and surrounding female spheres, I have not been able so far to confirm his statements by the application of staining reagents, but I feel assured that, were such nuclear staining successfully carried out, we should see in all essentials his clustered chromatic filament which, on breaking up into several filaments, forms, each with a certain amount of protoplasm, several flagella. His ideas as to the cause of the process are rather more hypothetical.

To return to the organism, immediately after fertilization, a process which when once recognized I have observed daily, it seems clear that the agitation of the pigment on the entrance of the flagellum is due merely to the mechanical motion of the wriggling flagellum, for it quiets down very soon. From this point on for ten to fifteen minutes it seems probable that, with suitable nuclear stains, one might demonstrate the coalescence of male and female nuclei, but I have not as yet done so. After a lapse of fifteen to twenty minutes the organism becomes motile, as above described, by the passage of the protoplasm into a conical process (Plate XII, Fig. 8), which gradually enlarges so as to contain the whole organism with the exception of a small spherical appendage into which most of the pigment is crowded (Plate XII, Fig. 9). The organism now appears quite fusiform, or at times egg-shaped, or even almost spherical, the anterior small end being free

from the pigment, which is distributed quite superficially over the posterior part. Toward the anterior part, but sometimes quite in the middle of the organism, there lies a fairly definite nucleus with well-marked nucleolus. This nucleus does not stain sharply, but is quite readily seen in fresh specimens, although no structure so definitely marked off by its different refractive index was observable in the adult male and female forms of the organism. The protoplasm retains the characteristic granular appearance seen in the female form.

Occasionally in beginning its movements away from the nucleus of the red corpuscle the organism leaves behind it part of the pigmented appendage—or it may possess two of these spherical appendages attached one behind the other, and in starting leave one of these behind to be broken down or engulfed by a leucocyte. The movement has nothing of the character of that of the flagellate forms, but is slow and even, the organism progressing invariably with the pointed end, free from pigment, forward. It can move in any direction readily, curving about obstacles and passing accurately between groups of corpuscles. Often it is seen to rotate continually on its long axis, at which times the superficial position of the pigment is readily seen. This rotation does not seem, however, to be in any way necessary to the forward motion. Again a third form of motion is observed in that a sort of peristaltic wavy constriction may repeatedly pass from anterior to posterior end of the organism, giving rise to very considerable distortions.

The forward progression of the organism occurs with very considerable force, as one may often be seen to thrust aside a group of red corpuscles. More often, however, they push directly through the obstacle, and it is interesting to observe that they can and do dash through the body of a leucocyte, tearing it apart, scattering its refractive granules into the plasma and often dividing its nucleus into two parts, thus leaving it to disintegrate. Passing on unharmed they attack masses of red corpuscles and leave those in their path as shadows of which soon only the nucleus is visible. This blanching of the corpuscles seems to become possible to them by the merest touch of their anterior pointed ends, which apparently puncture the capsule of the corpuscle and allow the hæmoglobin to escape into the plasma. Indeed,

in a thick slide of blood, a single organism may circle about and clear for itself a very considerable space in which there remain only shadows with the nuclei of corpuscles, and in some of the intense infections, which I have referred to, every slide of blood, after standing from thirty minutes to an hour, will show nothing but shadows, the organisms, very young and half-grown forms as well as segmenters, being often left free in the plasma. On one or two occasions these forms have been seen to put out pseudopods which attacked the corpuscles in a similar way.

In passing through a group of corpuscles it is frequently observed that the posterior end of the moving organism adheres to the corpuscles, and it may often be seen dragging three or four behind it. These soon become detached, however, and fall back, sometimes greatly distorted. In other cases the organism adhering to the corpuscle may draw out its capsule into a process, which gradually sinks back when the organism breaks away.

The ultimate fate and the true significance of these motile forms is difficult to determine, for in a slide, after being watched for more than one or two hours, the conditions become such that conclusions can scarcely be drawn as to the result under less artificial circumstances. Simple quieting down and final disintegration seem to be the end of many, but in some cases the organism was seen suddenly to stretch itself out by jerks into a greatly elongated form, sometimes dividing into segments connected by a fine thread. Generally in such cases the clear anterior end became separated from or connected only by a thread with the rest (Plate XII, Fig. 10), and often finally swam away by itself, leaving the rest to the leucocytes. This clear portion, however, was never seen to accomplish anything other than disintegration, although it was often watched with the idea that it might enter a corpuscle and set up a new cycle of existence.

It seems to me most reasonable to suppose that the motile form is the much sought resistant stage, or perhaps the first step in the production of such a stage. It seems probable that by the aid of such powerful means of locomotion and penetration it might make its way through a considerable obstacle of tissue to gain a new medium. This

of course might occur in several ways in the dead body subject to external violence. But in no case which I have examined post mortem did the organisms show any signs of life after the lapse of two to three hours, although the blood was still perfectly fluid in the vessels. In the living body, however, it seems that the intestine would form the most ready means of escape, and accordingly search was made in the fæces for such forms. In the birds at my disposal, however, in some cases the infection was so slight that results could hardly be expected, and in the cases where the infection was large, the presence of numerous echinostomes in the rectum rendered the mode of entry of the abundant blood corpuscles and organisms into the intestine doubtful. In dried smears from the contents of the small intestine, however, free organisms, free malarial pigment and one intra-corpuscular organism were found. More certain data might be expected from sections of the intestine with its contents, and examination of such sections showed immense numbers of organisms, mostly intra-corpuscular but sometimes extruded, in the capillaries of the stroma of the intestinal mucosa. In the mucous contents of the intestine were numerous *free* organisms, several of which exhibited the form described above as characteristic of the motile stage. The fact that the organisms were lying quite free in mucus, and that no corpuscles or nuclei of corpuscles were found, argues in favor of the idea that these organisms penetrated the mucosa after extrusion from the corpuscle. No organisms were found on the way through the epithelium. The inference that the organisms escape thus from the body seems plain, and further examinations of the intestinal contents obtained fresh immediately after death would be interesting.

In concluding this portion of the paper I may be allowed briefly to summarize what I have said.

Two types of organism occur so far as is known in the blood of birds. Minute morphological descriptions of these have been omitted in this paper. In the adult stage of one of these (Halteridium) there are certain differences between two forms which are thought to be sexual differences. Of these forms, one on extrusion from the corpuscle lies quiet and is soon approached and fertilized by a "flagellum" pro-

duced by the other form on extrusion from the corpuscle. The result of this fertilization is a motile stage which seems more resistant, and it is thought possible, in view of certain facts, that this form may make its way through the intestinal canal to the external world.

Reasoning by analogy it is thought very probable that the significance of the flagella in the human organism is similar to that given here for the organism of the birds.

THE PATHOLOGY OF HAEMATOZOAN INFECTIONS OF BIRDS.

The literature on the pathology of hæmatozoan infections is very meagre indeed. Danilewsky * speaks of the changes in the spleen and bone-marrow and describes macrophages with their varied contents. Kruse † made similar observations as to the spleen, bone-marrow, liver and lung, and Labbé ‡ also speaks in a general way of the gross changes in the organs. In the preceding paper on the subject, in this Journal (p. 103), prepared a year ago, I have given an account of the microscopic appearances of the various organs. I desire here to present a general summary of the more important pathological changes based upon the former observations and those made during the past summer.

Notwithstanding what has been said to the contrary, phagocytosis goes on very actively in the blood, as seen in a fresh slide. The leucocytes are seen to be contracted into small spheres on first examining the fresh drop of blood, but after about five minutes they begin to wander about and engulf whatever is foreign to the normal blood. These few minutes of inactivity are probably due to the shock of making the preparation. Both the eosinophiles and the rubinophiles (p. 113) show themselves to be very voracious. In the hardened tissues, however, these cells, which are readily recognized by their oxyphilic granulations, are very seldom seen to contain any pigment. On the other hand the cells most actively phagocytic there are principally the endothelial and connective-tissue cells. The deposition of pigment,

* *Ann. de l'Inst. Pasteur*, iv (1890), Parasitologie comparée, 1889.
† Virchow's *Archiv*, cxx and cxxi.
‡ *Arch. d. zool. expér.*, 1894.

together with the remains of infected corpuscles, organisms, etc., forms the main pathological change in the organs, and in order to make this change plain I shall begin by describing an organ in which it is most simple.

In the *kidney*, as in all the other organs, the capillaries and blood-vessels generally show a great number of infected corpuscles and some organisms extruded from the corpuscle. The epithelium of the tubules is in general fairly well preserved, although there are numerous instances of a hyaline degeneration of the cells. The glomeruli show nothing except the organisms within their capillaries. It is in the connective-tissue skeleton of the kidney with its contained capillaries that we see the main results of the infection. Many of the endothelial cells of the capillaries are much swollen and contain masses of pigment, yellow as well as malarial. Besides these there are cells belonging to the connective-tissue framework, which have become storehouses of pigment—large cells with large round or oval vesicular nucleus, which have become so loaded with pigment, etc., as to be very conspicuous in the section. No epithelial cells have been seen invaded.

In the *intestine* the connective-tissue cells of the stroma are phagocytic and sometimes contain considerable masses of pigment. Similar cells occur also in the muscular wall imbedded among the muscle fibres, the pigmentation being deepest in the subperitoneal portion. The endothelial cells of the vessels in this tissue are also pigmented. The epithelial cells of the mucosa never show foreign contents.

In the *bone-marrow* we have, besides fat cells and blood-vessels, cells large and small, which are generally spherical, with round nucleus and containing basophilic granulations, and other rounded cells and nuclei of various sizes with homogeneous protoplasm, all supported by a loose skeleton of connective tissue, made up of irregular, sometimes branching cells with pale ovoid nucleus. Of all these varieties of cells only the last are seen to be pigmented. Other large round cells do occasionally occur in the bone-marrow, however, which correspond closely to the red-corpuscle-carrying cells of the human spleen; these often contain several red corpuscles, some of which are infected.

In the *lung*, *brain*, and *pancreas*, although the capillaries contain many organisms, hardly any pigment deposit can be seen.

The thyroid and *adrenal*, however, show a very considerable pigmentation exactly corresponding in nature with that described for the kidney, the pigment being deposited in the endothelial and connective-tissue cells of the interglandular tissue, the secreting cells escaping. The adrenal, however, differs in that red-corpuscle-carrying cells occur in its small vessels together with large free pigmented cells with hyaline protoplasm and vesicular nucleus. These resemble precisely the macrophages in the spleen, to be described presently. In the tissue adjoining the thyroid gland there are large spaces lined by epithelioid cells, many of which contain pigment masses, or even several infected corpuscles. Lying free in these spaces are many large red-corpuscle-carrying cells together with numerous red corpuscles, leucocytes and cells desquamated from the walls, surrounded by a fine network apparently of coagulated fibrin. The nature of these spaces I do not know more exactly.

In the *pectoral muscle* in one case there was a greyish opaque spot which on section showed a mass of necrotic material lying in the muscle and surrounded by richly cellular tissue, which was quite deeply pigmented. The neighboring muscle fibres were much fragmented, with a proliferation of nuclei reminding one of that seen in the muscle in cases of trichiniasis. The tissue about the necrotic material had some of the characters of granulation tissue and was quite vascular, the elongated cells with large vesicular nucleus being the pigmented ones. Scattered about in this tissue were numerous giant cells with necrotic centre and many nuclei, such as are seen in miliary tubercles. Some of these were very large and without definite boundaries. The large mass of necrotic material was closely bordered by groups of clustered nuclei, suggesting that it had arisen from the coalescence of many giant cells. Through it there was scattered a good deal of malarial pigment. Whether all this diseased tissue in the muscle was due to the action of the hæmatozoon is doubtful; it seems to me much more probable that the deposit of malarial pigment throughout it was entirely secondary to its formation by some other process.

In the *liver* we have often a very extensive deposit of pigment. The liver cells themselves are never phagocytic, but the endothelial cells of the capillaries are often greatly swollen by their load of pigment and debris. In the blood-vessels, especially the branches of the portal vein, we find lying free the large macrophages, such as were seen to occur in the adrenal. These sometimes are so large as to contain masses of pigment equalling in size three or four liver cells. There are other phagocytic cells with large irregular nucleus which seem to lie between the liver cells and the endothelium and, although at first sight they have somewhat the appearance of liver cells, I am inclined to think them analogous to Kupffer's cells.

It is in the *spleen* that these processes reach their acme. This organ is almost always greatly swollen and blackened, the deposit of pigment being so disposed as to show a black network on section. The vascular pulp of the spleen is arranged in bands forming a network in whose meshes lie the lymphoid elements. The pigment deposit is confined to these vascular bands beginning at the margins, where they are sharply marked off from the lymphoid tissue, and gradually involving most of their width. The bands appear to be made up of a spongy reticulum of endothelial cells, between which the blood flows. These cells are more closely arranged at the boundaries of the lymphoid tissue, where they become conspicuous early in the infection by their pigmentary contents. In more intense infections a great majority of the fixed cells making up the pulp become loaded with pigment, and thus give rise to the black network mentioned above. It seems probable that such loaded cells may be washed into the blood stream and constitute the so-called macrophages. These have been mentioned as occurring in the blood of the liver and adrenal, and they are also seen in numbers in the veins of the spleen. They are cells varying greatly in size but generally of a somewhat rounded outline and with one or sometimes two vesicular nuclei; their protoplasm is generally very clear and is seen to contain a great deal of foreign material—yellow masses of pigment as well as the balls, irregular masses, and granules of malarial pigment, broken-down blood corpuscles and disintegrated nuclei, together with shrunken parasites. The last sometimes occur

in great numbers and form the only contents of the cell. In the spleen as elsewhere the endothelial cells of the vessel walls are often seen to be loaded with pigment. Red-corpuscle-carrying cells are not frequently seen.

The pigment found in all these cells is not exclusively that formed by the parasitic organisms, as has been mentioned. A large part of it is the iron-holding compound, or mixture of compounds, grouped under the name hæmosiderin, which are formed from the hæmoglobin set free on the rupture of the infected corpuscles.

We have, then, as the result of the hæmatozoan infections the production of one form of pigment by the parasites from the hæmoglobin, and of another form by the tissues of the host from the hæmoglobin set free into the blood. These solid pigments are stored up in the various organs, but more particularly in the spleen and liver, in cells belonging exclusively to the connective-tissue group which also engulf the infected corpuscles, broken remains of corpuscles and shrunken dead parasites. The anatomical changes, therefore, differ very slightly from those seen in the human being in malarial infections, so well described by Bignami * and by Barker,† the greatest difference perhaps lying in the less important part played by leucocytes in the case of the bird.

Finally, I wish to express my indebtedness to Dr. Thayer, of the Johns Hopkins Hospital, for his many kindnesses to me while engaged in this work, which was undertaken at his suggestion.

ADDENDUM.

Since the preparation of this article for the press I have examined the blood of a woman suffering from an infection with the æstivo-autumnal type of organism, in which a great number of crescents were to be seen. These in a freshly made slide of blood, with very few exceptions, retained their crescentic shape for only a few minutes. They soon drew themselves up, thus straightening out the curve of the crescent while shortening themselves into the well-known ovoid form.

* Bignami, *Bull. d. r. Accad. med. di Roma*, xix (1892-3).
† Barker, *Johns Hopkins Hospital Reports*, v (1895).

After the lapse of 10 to 12 minutes most of them were quite round and extra-corpuscular, the "bib" lying beside them as a delicate circle or "shadow" of the red corpuscle.

After 20 to 25 minutes certain ones of these spherical forms became flagellated; others, and especially those in which the pigment formed a definite ring and was not diffused throughout the organism, remained quiet and did not become flagellated. In a field where an example of each form could be watched, the flagella broke from the flagellated form and struggled about among the corpuscles, finally approaching the quiet spherical form; one of them entered, agitating the pigment greatly, sometimes spinning the ring about. The rest were refused admission, but swarmed about, beating their heads against the wall of the organism. This occurred after 35 to 45 minutes.

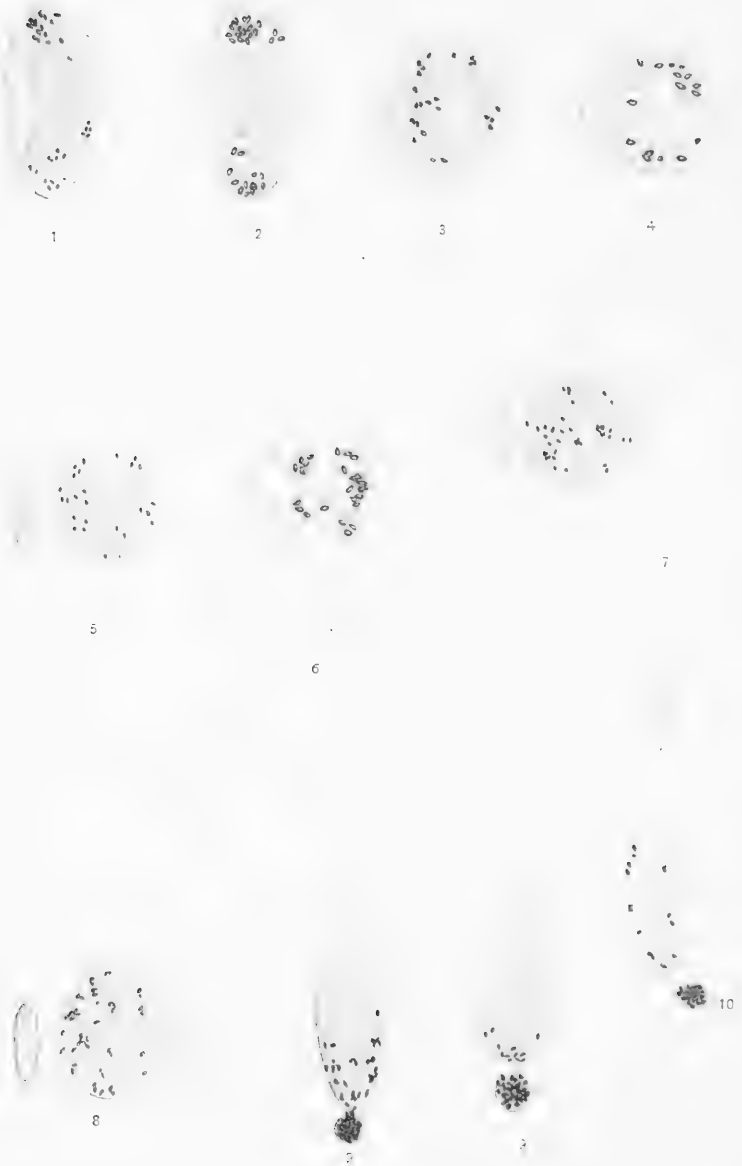
After the entrance of the flagellum the organism again became quiet and rather swollen, but although in the two instances in which this process was traced the fertilized form was watched for a long time, no form analogous to the "vermiculus" was seen.

This process has also been observed once by Drs. Warfield and Pancoast of the Johns Hopkins Hospital in another case of infection with the æstivo-autumnal parasite. It is evidently for the human being what was foreshadowed by the organisms of the bird.

DESCRIPTION OF PLATE XII.

All of the figures are of the Halteridium form of the parasite.

Fig. 1.—Adult granular, or female form.
Fig. 2.—Adult hyaline, or male form.
Fig. 3.—Adult female preparing for extrusion from the corpuscle.
Fig. 4.—Adult male preparing for extrusion from the corpuscle.
Fig. 5.—Adult female extruded and lying beside the nucleus of the corpuscle.
Fig. 6.—Flagellate form.
Fig. 7.—Process of fertilization.
Fig. 8.—First stage in formation of motile form.
Fig. 9.—Fully developed motile form or vermiculus.
Fig. 10.—Fully developed motile form in process of disintegration.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PRELIMINARY OBSERVATIONS ON A CASE OF PHYSIOLOGICAL ALBUMINURIA.

By TORALD SOLLMANN, M. D., and E. C. McCOMB.
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PLATE XIII.

Introductory. Usually physiological albuminuria is of the kind designated by Pavy as "cyclic," *i. e.* albumin in notable quantity is at times present, at times absent. This form has been frequently studied and the conditions influencing it are fairly well known. Instances of this kind are undoubtedly physiological.

The present case belongs to the much rarer form, which stands on the very boundary of the physiological and pathological. It was characterized by the constant presence in the urine of a considerable quantity of coagulable proteids, for a considerable length of time, without any other symptoms of general or renal disease.

In February, 1896, one of us accidentally discovered the presence of albumin in his urine. He was at the time in perfect health. A physical examination, made by Prof. C. F. Hoover, failed to disclose anything abnormal. Nor did the ophthalmoscope reveal any trace of albuminuric retinitis. Chemical tests made at intervals since that time have always shown albumin. Frequent microscopical examinations have failed to reveal casts. The state of health at the present date (January, 1898) is still perfect, and there are absolutely no other signs pointing to the presence of renal disease. The weight is 154 lbs., height 5 feet 8 inches, age 27 years.

General Considerations. In view of the comparative uncertainty of our knowledge of the mechanism of urinary secretion, it seems to us that any contribution which could possibly throw some light upon the question would be valuable. The study of the conditions which influence the quantity of the normal urinary constituents has already

yielded good results in this direction, but little has as yet been done in the way of making similar quantitative observations on the conditions influencing the excretion of proteids. The study of the present case therefore seemed to us of interest in this connection, as well as serving as a contribution to the knowledge of the different forms of albuminuria and the causes from which they result. In the present paper we content ourselves with giving the results of our work without attempting to apply them to the general questions suggested, as we are well aware that the number of observations is not sufficiently large nor are the results sufficiently unequivocal. The former defect we hope to remedy in the future. A table and a chart (Plate XIII) at the end of the article present the results of the observations.

The special questions which we attempted to answer are:

1. What is the nature of the proteids in the urine?

2. Is the albuminuria due to some general metabolic change or is it of renal origin?

3. What quantitative variations do the proteids undergo?

1. What is the Nature of the Proteids?

The proteid was found to consist of albumin and globulin. We at first attempted to determine the relative amount of these two substances, both by Pohl's and by Hammersten's method, but no satisfactory results were obtained, probably because the quantity of globulin was too small. We therefore contented ourselves with the estimation of the total coagulable proteid.

After the removal of the coagulable proteids the urine was frequently tested with sodium hydrate and cupric sulphate, but we could never obtain a biuret reaction, showing the absence of notable quantities of non-coagulable proteids.

2. Is the Albuminuria of Metabolic or of Renal Origin?

We attempted to answer this question by varying, as best we could, the conditions ordinarily accepted as influencing the general metabolism and the functional activity of the kidneys. Thus we examined the influence on the quantity of proteid excreted: *a*, of diet; *b*, of

temperature; *c*, of sleep; *d*, of diuretics; *e*, of drugs acting upon the circulation; *f*, of spontaneous variations in the volume of the urine.

a. The effect of diet. The fact that the person under observation eats a good deal of meat and little carbohydrate served to direct our attention to this point.

It will be seen from the tables and curves annexed that, on the whole,the proteids in the urine vary directly as the proteids in the diet, *e. g.* urinary proteids from February 9 to 12 (with a diet poor in proteids) average .5384 grm. per diem; from February 13 to 14 (with a diet rich in proteids), .6430 grm. On closer examination it will be seen that the changes in the urinary proteids only appear the day after the change in the diet. Taking this into account, we have for diet poor in proteids (February 10 to 12) .4465 grm.; for diet rich in proteids (February 14) .6748 grm. The average of the preceding week (with normal diet) was .5083 grm. And on the whole the curves of urea and of coagulable proteids from February 9 to 19 will be found to correspond very closely.

But on the other hand, when we tried to keep the diet approximately uniform in quantity and quality (from February 25 to March 4), large variations were seen in the urinary proteids, which do not at all correspond to changes in the amount of urea.

b. The effect of temperature. The influence of external temperature can be well studied from the chart, in which the average and in some cases the minimum daily temperatures are given (from records of the U. S. Meteorological Station, Cleveland). It will be seen that, as a rule, the coagulable proteids in the urine vary inversely as the temperature, and this in a very striking manner (January 23 to February 6; March 11 to 23; April 14 to 22). Equally striking is the steady decline in the average daily excretion per month from January to April (see below under 3).

Occasionally, however, there are changes in temperature without corresponding changes in the quantity of excreted proteids (April 7 to 14). Or the changes may even be in the same direction in both (February 6 to 8, April 25 to 27); but here the periods were only of two days, and the results could easily have been influenced by other

conditions which escaped observation. A cold sponge-bath of 10 minutes' duration (April 25 and 26) produced no rise in the amount of urinary proteids.

c. Influence of sleep. To determine the influence of sleep (rest and position?), we compared the average hourly excretion of proteids during the day and night, with the following results:

		Quantity of urine.	Quantity per hour.	Per cent. proteids.	Proteids per hour in grms.
April 6 to 7	Day	570 cc.	38 cc.	.0520	.0198
	Night	340	37.8	.0329	.01255
April 14 to 15	Day	950	67.9	.0402	.0273
	Night	370	37	.0557	.0206

It will be seen that during sleep the excretion of coagulable proteid is very notably diminished.

It would have been well to try the effect of severe exercise in this connection, but circumstances did not permit it at the time.

d. Influence of diuretics. In attempting to investigate the part which the kidneys played in the production of this albuminuria we tried (March 11 to April 9) the exhibition of various diuretics, viz. potassium nitrate, potassium acetate, caffeine, and urea, as a method of affecting the renal epithelium. On the whole these diuretics tended to cause a slight rise in the quantity of proteid excreted, but the effect was small and not always to be obtained. Even a dose of 31 grm. of potassium acetate produced very little change.

e. The influence of drugs acting upon the circulation. We chose trinitrin and digitalis for these experiments. The drugs were pushed until their effects were plainly felt. As will be seen from the curves, they tended to increase the excretion of proteid to some extent, but whether this action was due to changes in the renal circulation or to more general vascular effects, or to changes in the metabolism throughout the body or in some particular organ, the evidence at our command does not enable us to decide.

f. The relation between the quantity of urine and the quantity of proteids excreted. As we have seen, when the quantity of urine

is increased by diuretics that act chiefly on the renal epithelium there is little change in the quantity of excreted proteid, but what there is is in the direction of a rise. The same is true of changes produced by diuretics acting chiefly on the circulation. But for those changes in the volume of the urine which, for want of a better name, we may call spontaneous, the case appears to be different, the total proteids sometimes increasing as the quantity of urine diminishes, and sometimes varying directly as the quantity. Thus, in looking at the curves from January 29 to February 6, a period during which no drugs were administered, one cannot fail to be struck with the fact that the maxima of the proteid curve correspond with the minima of the curve representing the changes in the quantity of the urine. From January 25 to January 29, on the other hand, the two curves run parallel. It may be of interest to mention in this connection that the observed individual was unable to drink large quantities of liquid.

3. What Quantitative Variations do the Proteids Undergo?

As will be seen from the curves, the variations in proteids during the three months covered by our observations were comparatively small, much smaller than we expected. The quantity in the twenty-four hours fell once to .2592, and once to .3242, and rose once to .9010, and three times it lay between .814 and .855, but did not stay at any of these extremes longer than one day.

Excluding these exceptional results, we found that the proteid varied from .7 to .37 grm. in 24 hours.

The average in January (10 days) was .6616; February (23 days), .5419; March (16 days), .5111; April (18 days), .4651.

The average for the 67 days observed was .5318 grm. On the supposition, which appears legitimate, that the average daily output for each month was approximately the same as the average of the days actually observed in that month, the daily average for the four months over which the observations extended would be .5449.

SUMMARY.

1. The proteids consisted of albumins and globulins, varying in quantity between the extremes of .9010 grm. and .2592 grm. in the

24 hours, but usually between .7 and .37 grm., the average being .5317 grm.

2. The quantity of the proteids varies directly as the urea, inversely as the external temperature. The relation between the quantity of urine and the quantity of proteid is not constant. The quantity of the proteids is little affected by diuretics; it tends to be increased by certain drugs that act upon the circulation. It is lessened during sleep.

It is apt to suffer a sudden temporary increase, returning as suddenly to the usual average. For this phenomenon we have no explanation.

3. From .5449 to .6616 grm. of coagulable proteids a day (.0079 to .0094 grm. per kilo of body-weight) may be excreted through the urine for an indefinite time by an otherwise healthy individual without damage to either kidneys or organism.

NOTE.

The method employed for the estimation of proteids was the usual gravimetric method with heat precipitation. It was found that the addition of acetic acid, even when done as carefully as possible, detracted from the exactness of the result, and with these urines it was generally quite unnecessary. The method as carried out consisted in heating a certain quantity (usually 100 cc.) of the urine on the sand-bath, bringing to the boil about five times, filtering through ashless filter paper, again boiling the filtrate, cautiously adding a few drops of 2 per cent. acetic acid, and, if this produced any turbidity (which it rarely did), refiltering and repeating the process until all the proteid was precipitated. The precipitate was washed on the filter with distilled water until chlorine-free, then with alcohol, and was dried at 110° C.

As the weather was very cold during the greater part of the time, we usually analyzed the urines of two successive days at the same time. In a few cases we added a small quantity of salicylic acid. Most of the analyses were made on the mixed urine of 24 hours, but some on the mixed urine of 48 hours. All the determinations were made in duplicate, and of course the higher of the two accepted. The difference between the two determinations averaged in 52 cases 3 milligrammes per 100 cc.; in four cases it was between 10 and 11 mg. per 100 cc.

TABLE OF RESULTS.

Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.*	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
5 p. m. to 5 p. m. Jan. 19–20				(20)—3.3			
21				(21)—0.7			
22	.6045	930	27.5	—2.3		59.59	
23	.6045	930	27.5	—9.7		59.59	
24	.7357	880	28	—13.4		58.72	
25	.7357	880	28	—21.4		58.72	
26	.8397	1143	27	—18.7		69.21	
27	.8397	1143	27	—15.2		69.21	
28	.6000	750	29	—15.5		50.68	
29	.6000	750	29	—10.3		50.68	
30	.5279	1025	29	—8.4		69.26	
31	.5279	1025	29	—7		69.26	
Feb. 1	.4486	925	26	—2.8		56.05	
2	.4486	925	26	—2.3		56.05	
3	.6136	800	27	—2.3	23.276	50.72	
4	.6136	800	27	—2.3	23.276	50.72	
5	.4767	965	26	+0.3	29.734	59.05	
6	.4767	965	26	+1.9	29.734	59.05	
7	.4640	950	26	+0.4	28.781	57.56	
8	.4640	950	26	—1	28.781	57.56	
9	.8141	835	28	—1.7	27.791	54.47	Diet poor in proteids, rich in carbohydrates.
10	.4554	990	24	—1.8	20.766	55.36	Diet poor in proteids, rich in carbohydrates.
11	.4092	790	26	—3.3	20.648	47.86	Diet poor in proteids, rich in carbohydrates.
12	.4749	1060	23	—1.9	25.487	56.80	Diet normal in proteids, poor in carbohydrates.
13	.6111	970	24	—2.9	30.705	54.24	Rich in proteids, poor in carbohydrates.
14	.6748	1165	24	+1.9	34.281	65.14	Normal.
15	.7055	850	25	—0.2	30.517	43.49	Poor in both.

*Only the last two figures of the specific gravity, as expressed in the ordinary way, are given.

TABLE OF RESULTS.—*Continued.*

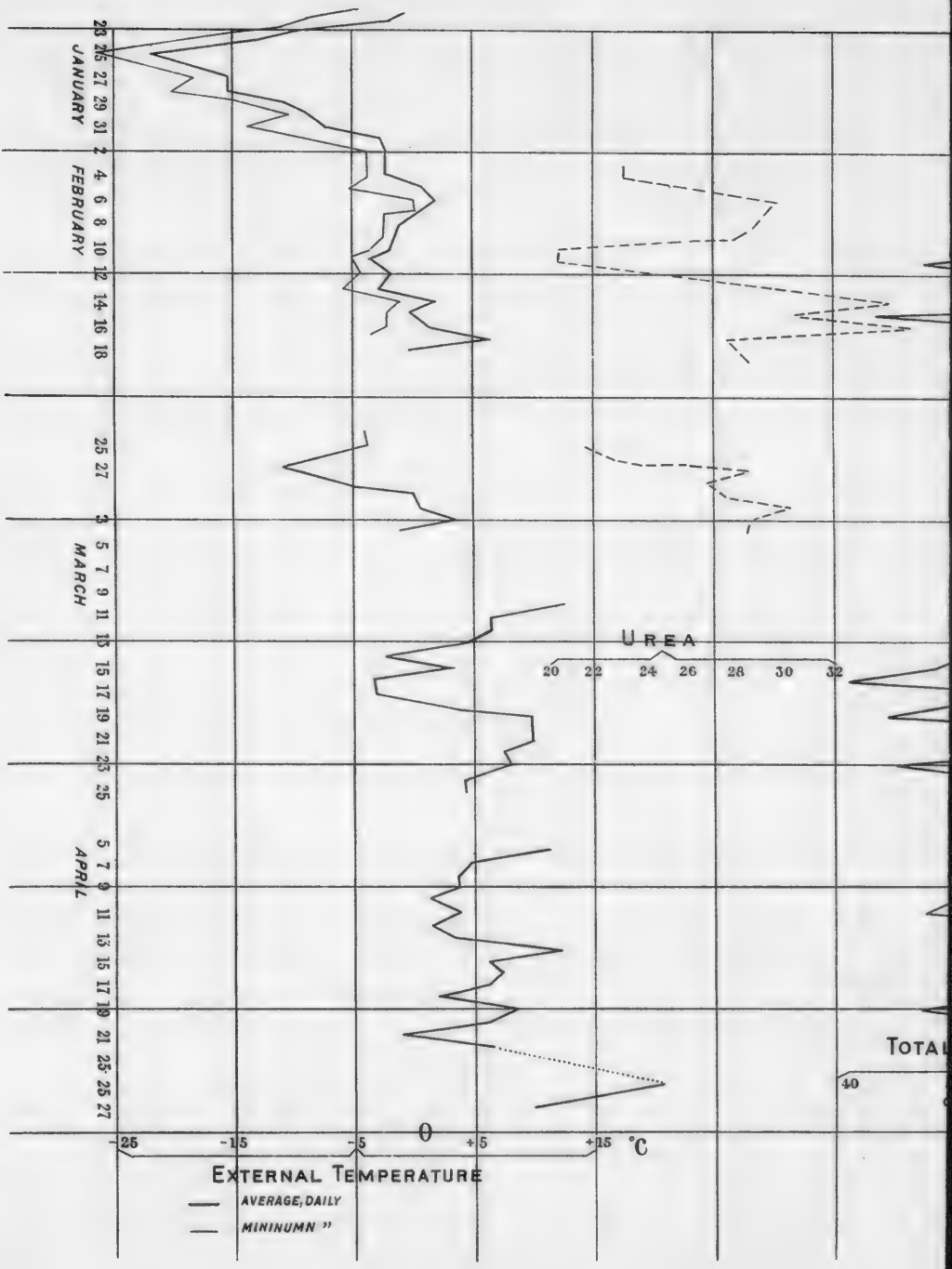
Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
5 p. m. to 5 p. m.							
Feb. 16	.5197	1120	22	(16)+1	35.189	57.41	Much H_2O taken.
17	.4233	1135	26	+6.6	27.523	68.76	Little " "
18	.4368	1050	26	—0.3	28.355	63.61	
19	.4368	1050	26		28.355	63.61	
25	.7040	950	23.5	—3.6	21.780	52.01	
26	.5251	960	26	—7.8	24.027	58.16	
27	.5330	1300	22	—10.9	28.449	66.64	
28	.7360	1150	23	—7.5	26.838	61.63	Amount of food and water taken practically constant.
to March 1	.9010	1070	23	0	27.459	57.34	
2		1080	27	0.5	30.332	67.94	
3	.4963	960	27	3.3	28.541	60.39	
4	.5184	800	27	—1.1	28.472	50.72	
8 a. m.				(9) 12.2			
to Mch. 11	.3242	860	27	(10) 6.7		54.10	
12	.4828	1210	22	6.7		62.02	4 grm. pot. nitrate taken.
13	.4922	850	27	4.4		53.48	
14	.8554	720	30	—2.2		50.33	
15	.6296	660	31	2.8		48.33	4 grm. pot. acetate.
16	.4015	550	32	—3.3		41.01	
17	.4905	780	30	—3.3		54.52	
18	.5420	825	27	1.6		51.90	.6 grm. caffeine.
19	.3660	630	30	10		44.04	
20	.2592	810	30	10		56.62	
21	.3003	910	26	10		55.13	2.1 grm. urea taken.
22		1090	25	7.2		63.49	.4 grm. urea "
23	.6541	690	28	7.8		45.01	

TABLE OF RESULTS.—*Continued.*

Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
7 a. m.							
to Mch. 24	.4633	820	28	(23) 2.2		53.50	
25		980	27	2.2		61.65	11.7 grm. pot. acet.
				(5) 11.1			
to April 7	.4093	910	27	4.4		57.25	
8	.4408	1520	22	3.3		77.91	31.1 grm. pot. acetate.
9	.4392	900	24	3.3		50.33	
10	.3999	930	23	1.1		49.84	
11	.3927	730	28	3.3		47.63	20 min. tr. digitalis.
12	.4407	920	28	1.1		60.02	
13	.3993	920	26	3.3		55.74	
14	.3696	1200	21	12.2		58.79	.0039 grm. trinitrin.
15	.5880	1320	23	6.1		73.82	" " "
16	.5852	950	26	7.2		57.55	.0033 " "
17	.4804	1570	20	6.1		73.16	" " "
18	.4970	1000	23.5	1.6		54.75	
19	.4520	800	25	8.9		46.60	
20	.5440	1000	25	6.1		58.25	[sulph. .0065 grm. strychnin
21		1010	24	—1.1		56.48	.0065 " " "
22	.5131	1010	25	6.7		58.83	
25	.5694	1010	26	21.1		61.19	
26	.4567	1015	24	15.6		56.78	Cold sponge-bath, about 10 min., morn. of 25th.
27	.3949	1010	25	10.		58.83	

DESCRIPTION OF PLATE XIII.

See the text.


JANUARY
FEBRUARY
MARCH
APRIL
UREA
20 22 24 26 28 30 32
TOTA
40
-25 -15 -5 0 +5 +15 °C
EXTERNAL TEMPERATURE
AVERAGE, DAILY
MINIMUM "

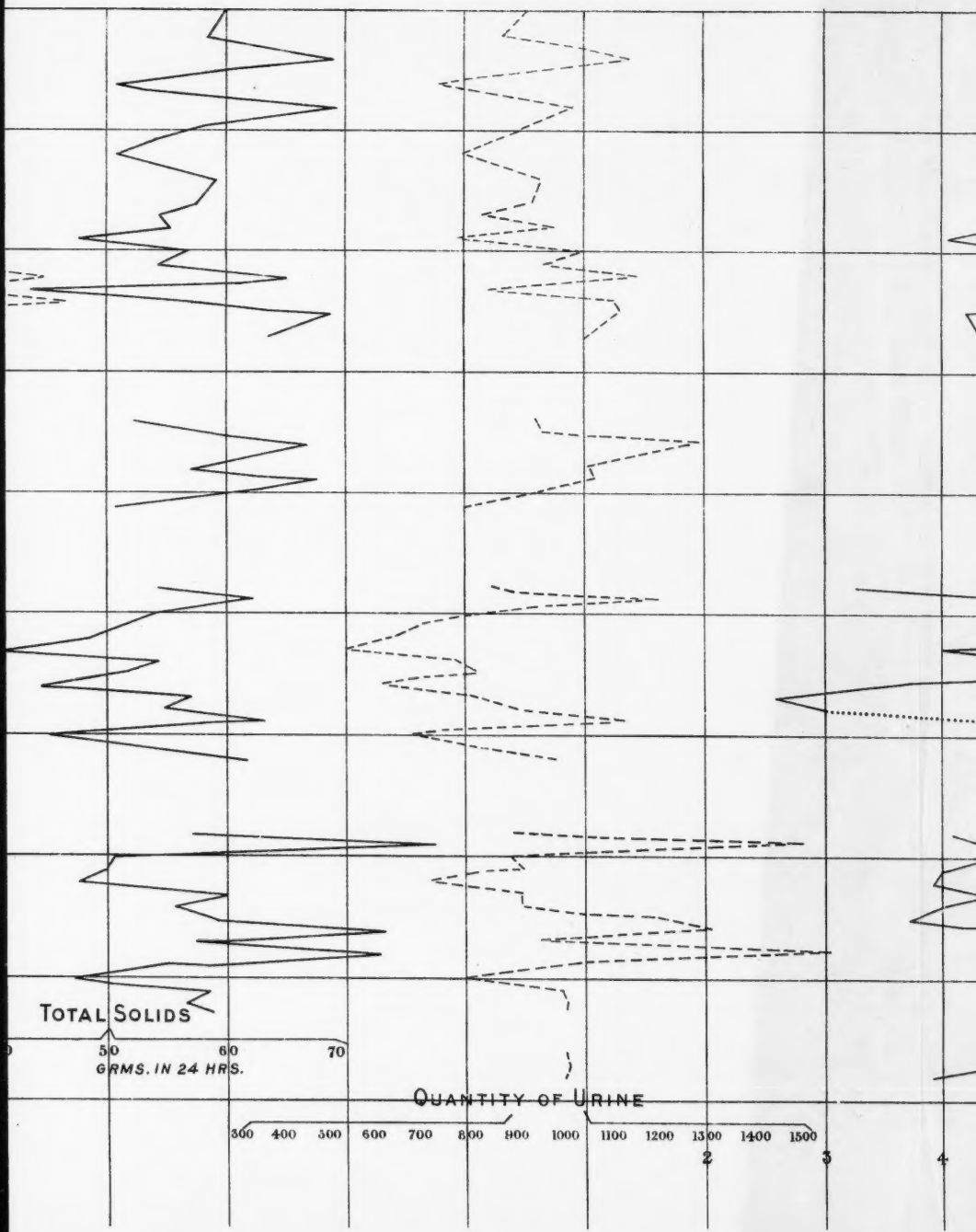
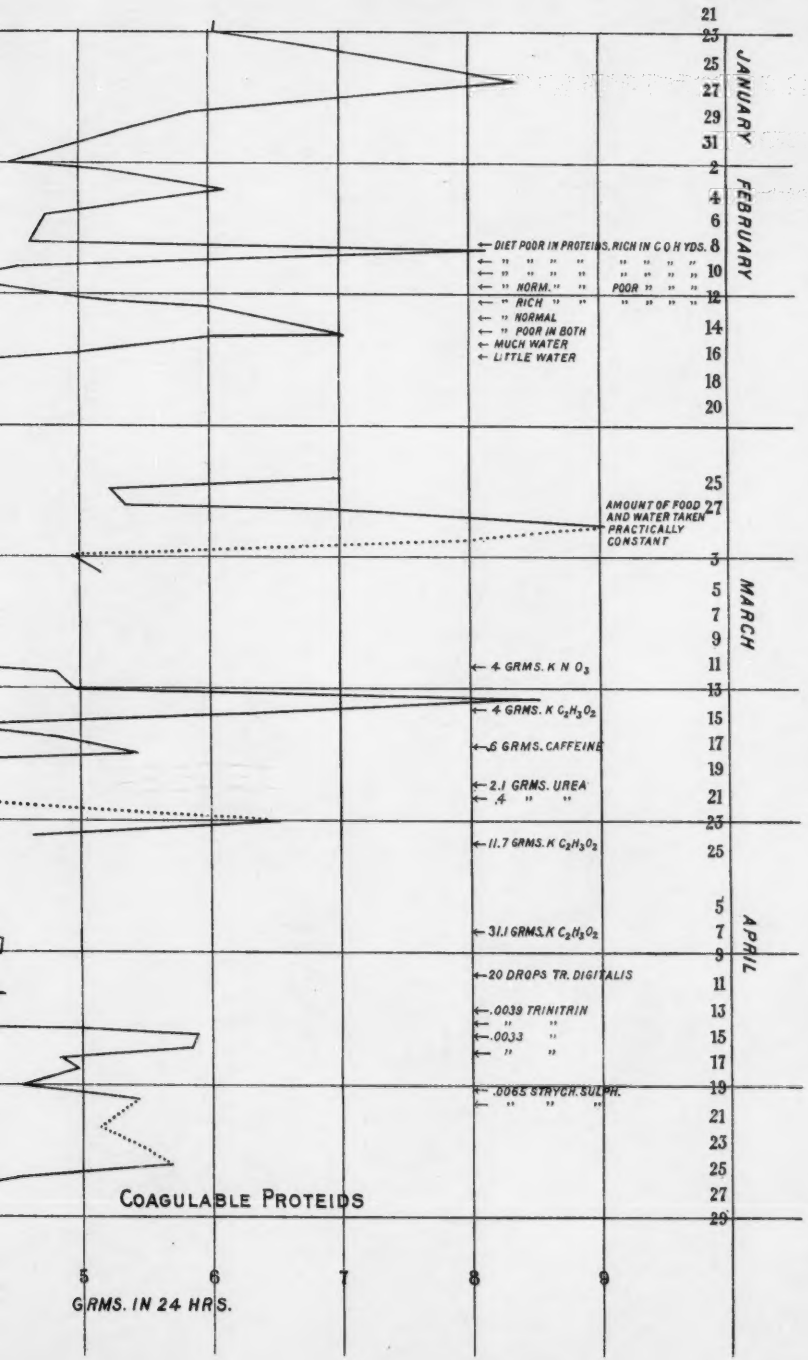

TOTAL SOLIDS
50
60
70
GRMS. IN 24 HRS.
QUANTITY OF URINE
300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
2
3
4

PLATE XIII.


21
23
25
27
29
31
JANUARY
2
4
6
8
10
12
14
16
18
20
FEBRUARY
← DIET POOR IN PROTEIDS. RICH IN C O H YDS.
← " " " " " " " " " "
← " " " " " " " " " "
← " NORM. " " POOR " " " "
← " RICH " " " " " "
← " NORMAL
← " POOR IN BOTH
← MUCH WATER
← LITTLE WATER
25
27
AMOUNT OF FOOD AND WATER TAKEN PRACTICALLY CONSTANT
3
5
7
9
11
13
15
17
19
21
23
25
MARCH
← 4 GRMS. K N O3
← 4 GRMS. K C2H3O2
← 6 GRMS. CAFFEINE
← 2.1 GRMS. UREA
← .4 " "
← 11.7 GRMS. K C2H3O2
← 31.1 GRMS. K C2H3O2
5
7
9
11
13
15
17
19
21
23
25
27
29
APRIL
← 20 DROPS TR. DIGITALIS
← .0039 TRINITRIN
← " "
← .0033 "
← " "
← .0065 STRYCH. SULPH.
← " " "
COAGULABLE PROTEIDS
5
6
7
8
9
GRMS. IN 24 HRS.